WEST Search History

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DATE: Tuesday, July 19, 2005

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,DWPI; PLUR=YES; OP=ADJ	
☐	L1	10/090326	1
☐	L2	multiplex PCR	1369
☐	L3	L1 and first amplification and second amplification	1
☐	L4	L1 and ((first same second) near (amplif$ or cycle$1))	1
☐	L5	L1 and ((different or multiple or plurality) same cycles)	1
☐	L6	L1 and (different temperature or different Tm)	1
☐	L7	multiplex near (PCR or polymerase chain reaction)	1464
☐	L8	L7 and (PCR cycles)	256
☐	L9	L8 and (different same (reaction near$3 condition))	0
☐	L10	(PCR or polymerase chain reaction or RT-PCR or reverse transcriptase Polymerase chain reaction or polymerase chain amplification)	97589
☐	L11	L10 AND ((MULTIPLE OR PLURAL$ OR DIFFERENT OR MORE) NEAR CYCLE$1)	2850
☐	L12	L10 AND (SEQUENTIAL NEAR CYCLE$1)	86
☐	L13	L10 AND ((DIFFERENT OR DISTINCT OR SEPARATE) NEAR (TEMPERATURE OR TM))	2421
☐	L14	((L11 OR L12 OR L13) AND ((FIRST NEAR$3 AMPLIFICATION)OR (FIRST NEAR$3 PRIMER SET) OR (FIRST NEAR$3 AMPLICON)))	0
☐	L15	((L11 OR L12 OR L13) AND (FIRST NEAR$3 AMPLIFICATION))	0
☐	L16	((L11 OR L12 OR L13) AND (FIRST NEAR AMPLIFICATION))	275
☐	L17	((L11 OR L12 OR L13) AND (FIRST NEAR PRIEMR SET))	0
☐	L18	((L11 OR L12 OR L13) AND (FIRST NEAR PRIMER SET))	50
☐	L19	((L11 OR L12 OR L13) AND (FIRST NEAR AMPLICON))	34
☐	L20	L19 and (second near amplicon)	17
☐	L21	L18 and (second near primer set)	42
☐	L22	(L16 and (second near amplification))	140
		DB=USPT,DWPI; PLUR=YES; OP=ADJ	
☐	L23	(L18 or L19 or L20 or L21 or L22) and (multiplex or multiplex amplification)	23
		DB=USPT,USOC,EPAB,DWPI; PLUR=YES; OP=ADJ	
☐	L24	GUS near$3 amplicon or 18S-rRNA near$3 amplicon or tyrosinase near$3 amplicon or CEA near$3 amplicon	0
☐	L25	reverse transcriptase polymerase chain reaction or RT-PCR	8037

☐	L26	L25 same PCR primer set	14
☐	L27	L26 same polymerase	3
☐	L28	L27 and (single tube or single vessel or single cartridge)	0
☐	L29	L27 and ((single tube) or (single vessel) or (single cartridge))	0
☐	L30	L25 and ((single tube) or (single vessel) or (single cartridge))	180
☐	L31	L25 same((single tube) or (single vessel) or (single cartridge))	55
☐	L32	((single tube) or (single vessel) or (single cartridge)) same L2	13
☐	L33	L32 and L25	3
☐	L34	L25 and cartridge	591
☐	L35	L34 and compartments	117
☐	L36	L35 and (vessel or reaction vessel)	46
☐	L37	L36 and automat$	32
	DB=PGPB,USPT,USOC,EPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L38	intraoperative PCR	2
☐	L39	(operation or surgery) same (PCR or polymerase chain reaction)	4363
☐	L40	L39 same threshold	49
☐	L41	L40 same indicator transcript	1
☐	L42	((operation or surgery)same patient) same (PCR or polymerase chain reaction)	335
☐	L43	L1 and indicator transcript	1
☐	L44	L43 and CEA transcript	0
☐	L45	L1 and CEA transcript	0
☐	L46	L1 and CEA	1
☐	L47	carcinoembryonic antigen	4080
☐	L48	L47 and L42	40
☐	L49	L48 and (during near$2 (surgery or operation))	0
☐	L50	L48 and (during same(surgery or operation))	24
☐	L51	L42 and (during same(surgery or operation))	138
☐	L52	((PCR or polymerase chain reaction)same (during same(surgery or operation)))	1058
☐	L53	L52 same L47	1
☐	L54	L52 same tumor antigen	0
☐	L55	L52 and L47	4
☐	L56	L52 and transcript	81
☐	L57	L52 same transcript	9
☐	L58	L52 same expression	32
☐	L59	L52 same threshold	6
☐	L60	L47 and L7	34
	DB=USPT,DWPI; PLUR=YES; OP=ADJ		

☐	L61	5837442.pn or 5882856.pn. or 5965710.pn. or 6057105.pn. or 6168948.pn. *DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ*	8
☐	L62	(two-stage near (PCR reaction or amplification reaction))	10
☐	L63	(two-stage near (multiplex PCR or multiplex amplification))	1
☐	L64	(two-stage and (multiplex PCR or multiplex amplification))	37
☐	L65	((RT-PCR or reverse transcriptase polymerase chain reaction) same (10 minutes))	1380
☐	L66	L65 same (single tube or single vessel or vessel or cartridge or tube)	123

END OF SEARCH HISTORY

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NEWS	4	FEB 28	BABS - Current-awareness alerts (SDIs) available
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NEWS	6	MAR 03	REGISTRY/ZREGISTRY - Sequence annotations enhanced
NEWS	7	MAR 03	MEDLINE file segment of TOXCENTER reloaded
NEWS	8	MAR 22	KOREAPAT now updated monthly; patent information enhanced
NEWS	9	MAR 22	Original IDE display format returns to REGISTRY/ZREGISTRY
NEWS	10	MAR 22	PATDPASPC - New patent database available
NEWS	11	MAR 22	REGISTRY/ZREGISTRY enhanced with experimental property tags
NEWS	12	APR 04	EPFULL enhanced with additional patent information and new fields
NEWS	13	APR 04	EMBASE - Database reloaded and enhanced
NEWS	14	APR 18	New CAS Information Use Policies available online
NEWS	15	APR 25	Patent searching, including current-awareness alerts (SDIs), based on application date in CA/CAplus and USPATFULL/USPAT2 may be affected by a change in filing date for U.S. applications.
NEWS	16	APR 28	Improved searching of U.S. Patent Classifications for U.S. patent records in CA/CAplus
NEWS	17	MAY 23	GBFULL enhanced with patent drawing images
NEWS	18	MAY 23	REGISTRY has been enhanced with source information from CHEMCATS
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NEWS	20	JUN 13	RUSSIAPAT: New full-text patent database on STN
NEWS	21	JUN 13	FRFULL enhanced with patent drawing images
NEWS	22	JUN 27	MARPAT displays enhanced with expanded G-group definitions and text labels
NEWS	23	JUL 01	MEDICONF removed from STN
NEWS	24	JUL 07	STN Patent Forums to be held in July 2005
NEWS	25	JUL 13	SCISEARCH reloaded

NEWS EXPRESS JUNE 13 CURRENT WINDOWS VERSION IS V8.0, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 13 JUNE 2005

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NEWS WWW	CAS World Wide Web Site (general information)

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FILE 'HOME' ENTERED AT 10:32:06 ON 19 JUL 2005

=> file .biotech caplus
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

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=> file .biotech caplus
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         7.12       7.33

FILES 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
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7 FILES IN THE FILE LIST

=> (RT-PCR or reverse transcriptase polymerase chain reaction)
(RT-PCR IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s (RT-PCR or reverse transcriptase polymerase chain reaction)
L1      301500 (RT-PCR OR REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION)

=> s l1 and (less (5a) 10 minute###)
L2           1 L1 AND (LESS (5A) 10 MINUTE###)

=> s l1 and 10 minut###
L3         153 L1 AND 10 MINUT###

=> s l3 and (single tube or tube or vessel or cartridge)
L4          13 L3 AND (SINGLE TUBE OR TUBE OR VESSEL OR CARTRIDGE)

=>
=> DUP REM L4
PROCESSING COMPLETED FOR L4
L5           13 DUP REM L4 (0 DUPLICATES REMOVED)

=> D IBIB ABS L5  1-13

L5    ANSWER 1 OF 13  BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-16354  BIOTECHDS
TITLE:            Detecting pathogen e.g. upper respiratory virus, by selecting
                  target sequence from pathogen, immobilizing
                  allelic-specific-oligonucleotide site onto solid matrix,
```

performing blot analyzes and comparing with control; SNP-specific DNA probe for upper respiratory virus pathogen detection

AUTHOR: TAM J W O; WOO H S; LO W S; SIA S F; FANG T K

PATENT ASSIGNEE: MEDICAL GENE CENT LTD; TAM J W O; WOO H S; LO W S; SIA S F; FANG T K

PATENT INFO: WO 2005038427 28 Apr 2005

APPLICATION INFO: WO 2004-US17767 4 Jun 2004

PRIORITY INFO: US 2003-476907 5 Jun 2003; US 2003-476907 5 Jun 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-333338 [34]

AN 2005-16354 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Detecting a pathogen, involves selecting target sequence from the pathogen, screening the sequence to obtain primers for amplification, selecting a probe from the sequence, immobilizing the allelic-specific-oligonucleotide site of each sequence onto a solid matrix for capturing target sequence, amplifying sequences, performing blot analyzes on sequence and comparing the results of the blot analysis using positive or negative controls.

DETAILED DESCRIPTION - Detecting (M1) a pathogen, involves selecting an appropriate target sequence from the pathogen, screening the sequence to obtain a set of appropriate primer(s) for amplification, selecting an appropriate probe from the sequence, immobilizing the allelic-specific-oligonucleotide site of each sequence onto a solid matrix suitable for capturing the target sequence, amplifying the sequence(s), where the immobilization and amplification are performed separately, performing blot analyzes on each sequence, and comparing the results of the blot analysis with using positive or negative controls. INDEPENDENT CLAIMS are also included for: (1) primer (I) used in (M1); (2) probe (II) used in (M1); (3) a kit for detecting pathogen comprising a compartment containing (I) or (II) or their combination; (4) a machine or computer capable of carrying out (M1); (5) a software dictating the complete or part of (M1); and (6) an array used in (M1).

BIOTECHNOLOGY - Preferred Method: In (M1), the sequence is a gene, pathogen or allelic sequence. The primer and the sequence-specific-oligonucleotide site are obtained by screening data from the GenBank or by sequencing the target sequence or in its combination. The target sequence comprises a gene, nucleic acid or DNA sequence. The probe is a single oligonucleotide. In (M1), more than one probe is selected. The probe is single nucleotide polymorphism probe. The solid matrix is a membrane. (M1) further involves amplifying the target sequence having a concentration less than 0.5 f moles, where the amplification step is performed by PCR, isothermal amplification, nested real-time PCR, or two-step nested real-time PCR. The amplification step comprises selecting an appropriate primer pair, performing reverse transcription on the primer pair, and performing the first and second round of PCR in a **single tube**, where more than one target sequence from a pathogen is amplified. The second round of PCR is performed with a thermal cycling program having the thermal cycling profile: 95 degreesC, 5 minutes; 95 degreesC, 10 seconds; 56 degreesC, 10 seconds; 72 degreesC, 30 seconds; 39 cycle repeats on steps 2-4, followed by 72 degreesC, **10 minutes** final extension time, or 95 degreesC, 3 minutes; 95 degreesC 10 seconds; 60 degreesC, 10 seconds (drop by 1 degreesC/cycle); 72 degreesC, 30 seconds; 9 cycle repeats on steps 2-4; 95 degreesC, 10 seconds; 56 degreesC, 10 seconds; 72 degreesC, 30 seconds; 9 cycle repeats on steps 6-8; and store at 4 degreesC. The second round PCR is done using 1 microl of the 1:10 dilution of the first round product in 50 microl reaction volume. The reverse transcription is performed with positive control RNA of 2 x 10-5 copies using the Thermoscript reverse transcriptase (**RT**)-**PCR** system and Platinum Taq DNA polymerase. In (M1), more than one pathogen and pair

of primers are used. (M1) is completed within two hours or within minutes, preferably within 20 minutes using enzyme link color development, or 2-3 minutes using colloidal gold or fluorescence dye. The solid matrix is a low-density array for detecting multiple severe acute respiratory syndrome (SARS) variant or a viral detection array for detecting upper respiratory tract infection. The array is on a single membrane. The steps of immobilization and amplification are performed sequentially or concurrently.

USE - (M1) is useful for detecting a pathogen which is an upper respiratory virus such as respiratory syncytial virus (RSV), parainfluenza virus type 1 (PIV1), PIV3, PIV3B, Mycoplasma pneumoniae (MP), Chlamydia pneumoniae (CP), Enterovirus (EN), influenza A (Inf A), Inf B, Adenovirus (Adeno), Metapneumovirus (Meta), and SARS coronavirus (SARS). (M1) is useful for identifying pathogens e.g. SARS causative pathogens (claimed). (51 pages)

L5 ANSWER 2 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-01408 BIOTECHDS
TITLE: Detecting and diagnosing a genetic material sample for e.g. diagnosing cancer, comprises using a multiplex real time polymerase chain reaction with primers but with their corresponding specific probe sets using the same reporter dye ;

polymerase chain reaction for use in disease diagnosis and mutation detection

AUTHOR: YEUNG W H A
PATENT ASSIGNEE: YEUNG W H A
PATENT INFO: US 2004229211 18 Nov 2004
APPLICATION INFO: US 2003-455043 22 Aug 2003
PRIORITY INFO: US 2003-455043 22 Aug 2003; US 2003-469812 13 May 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-821128 [81]
AN 2005-01408 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Detecting (M1) and diagnosing a genetic material sample by a multiplex real time polymerase chain reaction (PCR) technique with primers but with their corresponding probe sets using the same reporter dye, where the multiplex with the same one dye technique is referred to as MOD technology, and the primers are designed to target the same or different regions of the same gene in the sample.

DETAILED DESCRIPTION - Detecting (M1) and diagnosing any genetic material sample using a multiplex real time PCR technique with two or more primers but with their corresponding specific probe sets using the same reporter dye, where the multiplex with the same one dye technique is referred as MOD technology, and the primer sets are designed to target the same or different regions of the same gene in the biological sample, where the method can, if necessary, be followed by a confirmatory multiplex real time PCR with different probe dyes to determine the true composition and quantities of the different markers, where (M1) is in the form of a kit containing separately or in combination with specially designed nasal or throat swab tubes.

BIOTECHNOLOGY - Preferred Method: In (M1), the gene can be of two or more different genes in the biological sample. The gene or genetic material can be of any origin, DNA, RNA (using reverse transcriptase (**RT**)-**PCR**) or otherwise, so long it fits the criteria for the amplification purposes. The gene can be of any size or fragment. The gene is a cellular gene, an endogenous gene, transgene, or viral gene. (M1) Is not limited to real time PCR such as sybr green, TaqMan real time PCR or **RT-PCR**, but can be of any methodology, where the collective use of multiple markers with the same reporting agent is for the purpose of increasing sensitivity of the test. (M1) Is in the form of a diagnostic kit, consisting of different primer sets for

different regions of the same gene or different genes and the corresponding probes all in one reporter dye, regardless of mechanism and types. The diagnostic kit is with the same primer sets but with the corresponding probe sets with different reporter dyes, regardless of mechanism and types. The different primer sets for different regions of the same gene or different genes and the corresponding probes all in one dye and also in different dyes. The different primer sets target one of the genes of the severe acute respiratory syndrome (SARS) virus. The different primer sets target at least two to all of the genes of the SARS virus, namely the replicase polyprotein (Pol) region, the spike (S), membrane (M), and envelope (E) glycoprotein, and the nucleocapsid protein (N), or any yet discovered region.

USE - (M1) Is useful for detecting and diagnosing any genetic material sample using a multiplex real time PCR technique, detection and diagnosis of SARS infection carriers or patients. (M1) Is useful for confirmation, mutation status, staging, viral strain, viral quantity and prognosis of SARS patients, where the biological samples are from all the usual sources such as swab or lavage from nasal, nasopharynx and throat, blood plasma or serum, urine, stool, pleural or bronchial aspirate etc. The biological specimen comes only from the white blood cell or buffy coat of the blood sample or from the particle fractions of the blood, where RNA is associated. The detection and diagnosis is for other infectious diseases besides SARS. (M1) Is useful for the diagnosis of pre-malignant conditions or cancer, in the criminology field for ultra sensitive detection of minute or fragmented genetic materials, and in the detection of bio-terrorism materials with minute or fragments samples. (M1) Is useful in the field of use in animals instead of human, or in plants and agriculture (all claimed). (M1) Is useful for testing and sampling livestock for diseases such as sampling for SARS virus or H5N1 flu virus of livestocks, fresh or frozen food.

ADVANTAGE - (M1) Is very sensitive and enables to measure the viral load accurately thus leading to the prognosis of a patient and guide for treatment.

EXAMPLE - Cellular culture supernatant of severe acute respiratory syndrome (SARS) virus-infected Vero E6 cells was collected at the Chinese University of Hong Kong and RNA was extracted using a QIAamp viral RNA mini kit. The plasma or the buffy coat derived RNA extracted from the blood of SARS-patients. Blood (5 ml) was collected in EDTA tubes from patients. After centrifugation at 1600 g for **10 minutes** , the buffy coat layer was removed with phosphate buffered saline (PBS), spun down and the buffy coat layer was removed and transferred to a fresh micro-centrifuge **tube**, where it was re-suspended in 200 microl of PBS buffer. The process was similar for plasma except that the portion extracted was the plasma fraction of the blood and not the buffy coat. For the multiplex with the same one dye (MOD) methodology, the Pol 1 SARP probe used TET as the reporter dye. Primer sets were specifically designed to encompass the whole nucleocapsid region. Each probe was conjugated to the same reporter dye, (TET). This strategy was devised in order to increase the total signal generated by sub-genomic or low abundance PCR transcripts. Primers and probes designed to target the nucleocapsid region of the Tor2 isolate of SARS coronavirus. Multiplexing with one dye (MOD) technology gave a result that was approximately as many folds more sensitive than the normal real time PCR technology using only one primer set and probe as the number of different primers and probes put into the test. (15 pages)

L5 ANSWER 3 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-23134 BIOTECHDS
TITLE: Detecting or isolating liver stem cell, useful for developing artificial liver, involves using mRNA encoding integral membrane protein 2A as parameter, for detecting or isolating liver stem cell in sample;
stem cell detection and isolation for artificial organ

construction
PATENT ASSIGNEE: ZH KANAGAWA KAGAKU GIJUTSU ACAD; KIRIN BREWERY KK
PATENT INFO: JP 2004187679 8 Jul 2004
APPLICATION INFO: JP 2003-350081 8 Oct 2003
PRIORITY INFO: JP 2002-343950 27 Nov 2002; JP 2002-343950 27 Nov 2002
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2004-493008 [47]
AN 2004-23134 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Detecting or isolating (M1) liver stem cell, involves using mRNA encoding integral membrane protein 2A (Itm2A) or Itm2A protein as a parameter, for detecting or isolating liver stem cell in a sample, where (M1) involves using monoclonal antibodies for carrying out antigen antibody reaction with Itm2A in the extra cellular region, and the isolation is carried out under in vitro conditions.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is included for nucleic acid (I) hybridizing with mRNA or cDNA of Itm2A, for measuring mRNA or cDNA of Itm2A.

BIOTECHNOLOGY - Preferred Method: In (M1), the monoclonal antibody of Itm2A is made to react with cell having Itm2A in the extracellular region, and the monoclonal antibody coupled to the cell surface is used as a parameter for detecting liver stem cell. The existence of mRNA encoding Itm2A is taken as a parameter for detecting liver stem cell. (M1) involves detecting or isolating liver stem cell from cell population of a fetal liver or adult liver during regeneration, where hepatotoxin is administered into adult liver during regeneration, and a portion of the liver is cut. (M1) also involves detecting or isolating liver stem cell contained in the tissue other than liver or from the cell population of an embryonic stem cell. The Itm2A is a human Itm2A. Preferred Nucleic Acid: (I) is a probe or a primer.

USE - (M1) is useful for detecting or isolating liver stem cell from cell population of fetal liver or adult liver during regeneration (claimed). (M1) is useful in organ transplantation, and in the development of artificial liver.

ADVANTAGE - (M1) enables separation and recovery of liver stem cells, with high purity.

EXAMPLE - Liver was extracted from C57/BL6 mouse. The liver was digested by collagenase processing, and a cell suspension was prepared. Next, sequential reaction of the cell suspension was carried out with hamster anti-delta-like (dlk) monoclonal antibody, biotinylated anti-hamster antibody and streptoavidine bead. An auto-magnetic cell sorting (MACS) was performed. Thus, mouse embryonal hepatocytes were prepared. The 2-acetylaminofluorene was orally administered for four days at dosage of 1.5 mg/kg body weight to the F344 male rat. A partial hepatectomy was performed. Liver was extracted on day 0, 4, 7, 9, 11, 13 and 20. The laparotomy was performed. The liver was treated with 0.05% of collagenase solution for **10 minutes**. The digested liver was collected in 50 ml centrifuge **tube**, and centrifugation was carried out for 1 minute. The RNA was extracted from the liver using Trizol reagent. The cDNA was synthesized using the extracted RNA. Then, the expression of integral membrane protein 2A (Itm2A) was analyzed by PCR, using primers having sequences such as 5'-atttaccatggtgagatgtg-3' and 5'-aagtgtctaatcttccagca-3'. From gene expression analysis, by Northern blotting and reverse transcriptase (**RT**)-**PCR**, Itm2A was found to be expressed in embryonal hepatocytes. The presence of Itm2A was further confirmed by using anti-Itm2A antibody, and thus a liver stem cell was identified using the Itm2A gene or Itm2A protein. (14 pages)

L5 ANSWER 4 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-20756 BIOTECHDS
TITLE: Eliminating redundant sequences and identifying unique

sequences from samples, by isolating RNAs/DNAs from samples, generating cDNA, hybridizing cDNAs with RNAs, degrading hybridized complements, amplifying cDNAs/RNAs;
for use in redundant DNA sequence elimination

AUTHOR: IQBAL S; CHIN R C
PATENT ASSIGNEE: IQBAL S; CHIN R C
PATENT INFO: US 2003064373 3 Apr 2003
APPLICATION INFO: US 2001-961089 24 Sep 2001
PRIORITY INFO: US 2001-961089 24 Sep 2001; US 2001-961089 24 Sep 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-567177 [53]
AN 2003-20756 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Eliminating redundant sequences using biomolecular degrading enzymatic reagents and radiolabeled/fluorescent dye assay and identifying unique sequences from two or more samples, comprising isolating RNAs/DNAs from samples, generating cDNA from RNAs, hybridizing complementary cDNAs with RNAs, degrading hybridized complements, amplifying resultant cDNAs/RNAs (I), displaying and reading (I), and sequencing (I), is new.

DETAILED DESCRIPTION - Eliminating (M1) redundant sequences which are common between two or more samples using biomolecular degrading enzymatic reagents and radiolabeled assay or fluorescent dye assay to identify the remaining sequences which are uniquely expressed or over and under expressed in these samples, comprising: (a) isolating RNAs or DNAs from samples which are of interest; (b) generating cDNA from RNAs using reverse transcriptase (**RT**)-**PCR** methods and technologies; (c) cross mixing complementary cDNAs with RNAs of samples of interest; (d) degrading the hybridized complements using degrading reagents, amplifying resultant cDNAs or RNAs by PCR amplification; (e) displaying and reading resultant cDNAs or RNAs; and (f) sequencing cDNAs or RNAs. An INDEPENDENT CLAIM is also included for degrading (M2) cDNA/RNA, cDNA/cDNA or RNA/RNA hybrids using Si nuclease enzyme or Exonuclease enzyme to eliminate common cDNA's or RNA's from two different cell and tissue types.

BIOTECHNOLOGY - Preferred Method: The samples of interest are cells, tissues, pathogens, plants and animals, and RNAs and DNAs are isolated using standard prior art methods and technologies. The samples are comprised of cells, tissues, pathogens, plants and animals with little or totally known genetic sequence information, or which have differentiated due to a diseased state, developmental change, or induced by an external or internal stimulus. The **RT-PCR** methods and technologies is comprised of radioactive labels or fluorescent dyes labeled to anchor oligo-dT primers to generate cDNAs or RNAs. The fluorescent dyes absorb and fluoresce at two distinct wavelengths, are fluorescence quencher pairs, fluoresce at two distinct fluorescent lifetimes or at two distinct polarizations. Cross mixing complementary samples comprises mixing cDNAs and RNAs from the above samples, forming hybridized cDNA/RNA. The degrading reagents are Exonuclease III or VII enzyme. Amplifying the PCR comprises of using AmpliTaq Gold Polymerase. The resultant cDNAs or RNAs are displayed by electrophoresis gel or capillary electrophoresis. The resultant cDNAs or RNAs are read by photographic plate or scanning fluorescence spectrophotometer. The cDNAs or RNAs are sequenced by a MegaBase DNA sequencer or similar automated sequences.

USE - M1 is useful for eliminating redundant sequences which are common between two or more samples and for identifying the remaining sequences which are uniquely expressed or over and under expressed in these samples (claimed). M1 is useful for identifying and isolating different genes expressed between diseased and normal tissues for use in discovering new drugs. M1 is useful for isolating differentially expressed genes or gene fragments and to isolate and identify medium to low gene expressions that may otherwise be buried under gene noise.

ADVANTAGE - M1 efficiently and cheaply identifies and accurately isolates different genes expressed between diseased and normal tissues. M1 can be implemented in a single reaction **tube**, is amendable to miniaturization, and is extremely cost- effective which is of benefit in toxicogenomic and drug discovery applications.

EXAMPLE - Reverse transcription protocol was performed using dH2O, 5X reverse transcriptase (RT) Buffer, dNTP (250 micro-M), H- T11 M (2 micro-M), Total RNA (0.1 micro-g/micro-l), and moloney murine leukemia virus (MMLV) RT. Following the RT reaction, an RNAse H incubation was performed by adding 1.0 microl of RNAse H (4 U/yl) to the 20yl RT reaction product comprising dH2O, 5X HT Buffer, dNTP (250 nuM), H-T11M (2 micro-M), cDNA/mRNA complex (0.01 micro-g/ml), MMLV RT and RNAse H. At this point the foreign cDNA was ready to be mixed with the foreign RNA. Degrading reagents such as Exonuclease III (digests double stranded (ds)DNA and portions of the DNA/RNA complex) or Exonuclease VII (digests single stranded (ss)DNA) were used. The product of the preferential display reactions was followed by a PCR. For the Exonuclease VII approach 0.2 microl of P33ATP, 2.0 microl of H-APX and H-T11M were added to the preferential display product prior to the PCR reaction. The Exonuclease III approach did not require these components, instead 2.0 microl of H-APX, 20 microl of H-T11-M, 0.2 microl of P33ATP, 1.6 microl of dNTP (25 microM), 1.2 microl of 10X PCR Buffer and 2.2 microl of dH2O were added. These made a 20 microl total reaction volume for PCR. Genes display was carried out using QuickPoint (RTM) Gel system. The precast gel was pre-electrophoresed for 5 minutes. The PCR reaction was mixed with a QuickPoint sample loading buffer and was then heated to 80 degreesC for 2 minutes and was loaded immediately onto the gel (6 % polyacrylamide). The sample loading buffer was loaded onto the remaining wells to assure a straight banding pattern. Electrophoresis was carried out in QuickPoint (RTM) cell at 1200 V for approximately **10 minutes** depending upon the length of the sequences. Following electrophoresis, the glass cassette enclosing the gel was washed in water for 5 minutes on a low speed shaker, dried on an oven at 80 degreesC for 20 minutes and exposed to X-ray film overnight to generate an autoradiogram. (10 pages)

L5 ANSWER 5 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-10537 BIOTECHDS
TITLE: Detection of a breast cancer cell by detecting gene abnormalities;
for use in mamma cancer diagnosis
PATENT ASSIGNEE: SUMITOMO CHEM CO LTD
PATENT INFO: JP 2003284596 7 Oct 2003
APPLICATION INFO: JP 2002-94487 29 Mar 2002
PRIORITY INFO: JP 2002-94487 29 Mar 2002; JP 2002-94487 29 Mar 2002
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2004-126224 [13]
AN 2004-10537 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Detection (M1) of a breast cancer cell comprising: (a) measuring the expression level in the test substance of the 1 or more genes chosen from the gene which has a specific base sequence, or its ortholog; and (b) comparing the measured value of the expression level of the gene obtained with the control contrast value of the expression level of the gene, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) evaluation (M2) of anti- cancer activity of a material comprising: (a) breast cancer tissue or a breast cancer cell, and a test material are made to contact (b) measuring the expression level of the 1 or more genes chosen from the gene or its ortholog which has the base sequence registered into GenBank in any one of the registration number in a test material, the tissue which made it contact, or a cell shown by the registration-number group 1 or 2; and (c) comparing the measured value of

the expression level of the gene obtained with the control contrast value of the expression level of said gene, and evaluates anti- cancer activity of a test material based on the difference; (2) a search method of an anti- cancer active substance; (3) antineoplastic drug which contains as an active ingredient the anti- cancer active substance selected; (4) evaluation (M3) of carcinogenesis activity of a material comprising: (a) contacting the tissue or the cell derived from a breast, and a test material; (b) measuring the expression level of the 1 or more gene chosen from the gene or its ortholog which has the base sequence registered into GenBank in any one of the registration number in a test material, the tissue which made it contact, or a cell shown by the registration-number group 1 or 2; (c) comparing the measured value of the expression level of the gene obtained with the control contrast value of the expression level of the gene; and (d) evaluating carcinogenesis activity of a test material based on the difference;

BIOTECHNOLOGY - Preferred Method: The method is characterized by containing the process which detects the breast cancer cell in the test substance based on the variant. Measurement of the expression level of a gene is made by measurement of the transcription or transfer amount of resources of the gene. The control contrast value of the expression level of a gene is a value of the expression level in the normal tissue or normal cell of the gene. In detection of a breast cancer cell (M1): (a) the measured value of the expression level of the gene or its ortholog which has the base sequence registered into GenBank in any one of the registration number shown by the registration-number group 1 is higher than a control contrast value be index; (b) making into an index for the measured value of the expression level of the gene or its ortholog which has the base sequence registered into GenBank in any one of the registration number shown by the registration-number group 2 to be lower than a control contrast value. The control contrast value of the expression level of a gene is a value of the expression level in the breast cancer tissue or the breast cancer cell of said gene. In M2, (a) the measured value of the gene or its ortholog which has the base sequence registered into GenBank in any one of the registration number shown by the registration-number group 1 is lower than a control contrast value be index; and (b) evaluate anti- cancer activity of a test material by making into an index for the measured value of the expression level of the gene or its ortholog which has the base sequence registered into GenBank in any one of the registration number shown by the registration-number group 2 to be higher than a control contrast value. In M3: (a) the parameter index for the measured value of the gene which has the base sequence registered into GenBank in any one of the registration number shown by the registration-number group 1, or its ortholog to be higher than a control contrast value; and (b) evaluating carcinogenesis activity of a to-be-tested substance by making into a parameter index for the measured value of the gene which has the base sequence registered into GenBank in any one of the registration number shown by the registration-number group 2, or the expression level of the ortholog to be lower than a control contrast value; Preferred Gene: The gene by which an expression level is measured in this invention is a 1 or more gene chosen from the gene which has the base sequence registered into GenBank in any one of the registration number shown by said registration-number group 1 or 2, or its ortholog (Hereafter, it may be collectively described as this gene.). The information (for example) related to the base sequence registered into GenBank can acquire by searching based on said registration number (Accession Number) assign provided to each gene by National Center for Biotechnology Information WEB page (URL;http://www.ncbi.nlm.nih.gov) to GenBank. The gene which has the base sequence which deletion of the base by the variation mutation produced natural according to the variant between the species difference of an organism, individual differences or an organ, and a structure tissue etc., substitution, or addition produced is also contained in the base sequence of said gene besides (which has the completely same base

sequence as said well-known base sequence) a gene by this gene utilized in this invention. Preparation: mRNA is prepared from the structure tissue which is going to measure the expression level of this gene, or a cell by the method described in above (1 the northern hybridizing method), and the same method. Measurement of the expression level of this gene comprises preparation of total RNA by using a human breast cancer structural tissue and each normal tissue, adding and freezing 10ml TRIZOL reagent. This is homogenised and left at room temperature for 5 minutes. Then **10 minutes** and 9,000 rpm at 4 degrees C of aqueous layers and is collected and covered in 50ml centrifugation **tube** after centrifugation. The chloroform (Kanto Kagaku make) of the 1/5 capacitance volume ofTRIZOL Reagent is added, after stirring vigorously vertically for 15 seconds,. it is left at 5 minutes room temperature. Subsequently, **10 minutes**, 9,000 rpm of 4 degrees C of aqueous layers is collected, covered in new 50 ml centrifugation **tube**, after centrifuging. Furthermore, 2-propanol (Kanto Kagaku make) of the 1/2 capacitance volume of TRIZOL Reagent is added, ten minutes is stood at room temperature after an inversion mixture. After 4 degrees C, 9,000 rpm, **10 minutes**, and a centrifugation, a supernatant liquid is removed and a pellet is obtained. The obtained pellet is dissolved with the DEPC processing sterilization distilled water (Wako Pure chemical Co., Ltd. company make) of the 1/10 capacitance volume of TRIZOL Reagent. RLTbuffer (A 10 micro-L(beta)-mercaptoethanol / mL RLT buffer) 350 micro-L of RNeasy Mini Kit (product made from QIAGEN) attachment is added (0.1 ml / of RNA solutions obtained), and mixed. Furthermore, 250-micro-L ethanol (Kanto Kagaku make) is added. Then, a column is moved to a new Eppendorf **tube** and 50 micro-L of distilled water of RNeasy Mini Kit attachment is added, it stands at 1 minutes room temperature. It elutes by room temperature, 10,000 rpm, 1 minutes, and centrifugation after still-standing, total RNA is obtained. Operation similar to the above is performed also with a normal tissue, and total RNA is prepared.

USE - The methods are useful for detecting breast cancer (claimed).

EXAMPLE - Anti- cancer activity evaluation using quantitative **RT-PCR** comprises preparing a cell firewood per 6 well plate (Sumitomo bakelite company make) 1 well about the culture cultivation cell derived from a breast cancer. The culture occurs at 37 degrees C and 5-% carbon-dioxide existence using a FBS -containing culture medium. Subsequently, 2 hours after exchanging for the FBS -containing culture medium which added the solution formed by dissolving a to-be-tested substance for a culture medium in DMSO 0.1%, or 48 hours cultivating. The cell culture liquid of a to-be-tested-substance addition division is obtained. On the other hand, the cell culture liquid of a to-be-tested-substance additive-free division is prepared by culture cultivating in the same manner to the above except using DMSO 0.1% instead of the to-be-tested-substance solution. After preparing total RNA from a different two or more biopsy sample which contains the biopsy sample derived from a healthy subject, fragment-ized cRNA derived from the biopsy sample derived from a healthy subject and fragment-ized cRNA derived from a test biopsy sample are prepared. Next, it dye stains, after hybridizing fragment-ized cRNA derived from the biopsy sample derived from a healthy subject, and fragment-ized cRNA derived from a test biopsy sample by the same operation to the probe array to which the oligonucleotide which has the partial base sequence of the base sequence of this gene was fixed. The obtained dyeing finished probe array is used for to HP GeneArray Scanner (product made from Affymetrix). A signal is read by measuring a 570 nm fluorescent brightness, and the comparison analysis of the obtained result is carried out by GeneChip Microarray Suite (product made from Affymetrix). The gene which has the base sequence registered into GenBank in any one of the registration number shown by the registration-number group 1 or 2, or the expression level of the ortholog is compared between the biopsy sample derived from a healthy subject, and a test biopsy sample, Based on the variant, the breast

cancer cell in a test biopsy sample is detected. That is, if the measured value of the expression level in the gene which has the base sequence registered into GenBank in any one of the registration number shown by the registration-number group 1, or the test biopsy sample of the ortholog is higher than the expression level of said gene in the biopsy sample derived from a healthy subject, it will test that a breast cancer cell exists in said test biopsy sample. Moreover, if the measured value of the expression level in the gene which has the base sequence registered into GenBank in any one of the registration number shown by the registration-number group 2, or the test biopsy sample of the ortholog is lower than the expression level of said gene in the biopsy sample derived from a healthy subject, it will test that a breast cancer cell exists in said test biopsy sample. (50 pages)

L5 ANSWER 6 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-09678 BIOTECHDS
TITLE: Co-detecting Vitivirus, Foveavirus, Closterovirus genus in samples, by performing reverse transcription-PCR using degenerate primers that amplify regions of Vitivirus, Foveavirus, and Closterovirus, followed by nested PCR; polymerase chain reaction for use in virus detection
AUTHOR: DOVAS C
PATENT ASSIGNEE: VITRO HELLAS SA
PATENT INFO: EP 1371736 17 Dec 2003
APPLICATION INFO: EP 2002-386008 4 Jun 2002
PRIORITY INFO: EP 2002-386008 4 Jun 2002; EP 2002-386008 4 Jun 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-111194 [12]
AN 2004-09678 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Co-detecting members of Vitivirus, Foveavirus and Closterovirus genus in sample, involves isolating RNA from sample, carrying out nested reverse transcription-PCR comprising amplifying using degenerate primers that amplify part of polymerase region of Vitivirus and Foveavirus and part of HSP 70 homolog gene of Closterovirus in first-stage reverse transcriptase-PCR, followed by nested PCR amplification, and analyzing reaction products, is new.

DETAILED DESCRIPTION - Co-detecting (M1) members of Vitivirus, Foveavirus and Closterovirus genus in a sample suspected of being infected with the viruses, involves: (A) isolating RNA from the sample suspected of being infected with one or more the viruses, or extracting RNA from the sample spotted in a nylon membrane; (B) performing a nested reverse transcription-polymerase chain reaction to selectively amplify a targeted nucleotide sequence within the isolated RNA, the nested reverse transcription-polymerase chain reaction comprising the steps of: (i) amplifying using degenerate oligonucleotide primers in a first-stage reverse transcription polymerase chain reaction (**RT-PCR**) mixture, first targeted nucleotide sequences, the first targeted nucleotide sequences which comprise: (a) a 363 base-pair region of the polymerase gene from members of Vitivirus and Foveavirus genus; or (b) a 580-620 base-pair region of the heat shock protein 70 (HSP 70) homologue gene from members of Closterovirus genus, or both (a) and (b), where the oligonucleotide primers specific for members of Vitivirus and Foveavirus genus comprise: Forward primer: 5'-WGCIAARGCIGGICARAC-3' (S1), Reverse primers: 5'-RMYTCICCISWRAAICKCAT-3' (S2), and 5'-GCCGSWRAAGCKCAT-3' (S3), and where the oligonucleotide primers specific for members of Closterovirus genus comprise: Forward primers: 5'-GGIHTIGAITTYGGIACIACITT-3' (S6), and 5'-GTTYGGGACGACGTT-3' (S7), Reverse primer: 5'-GTICCICCICCNAARTC-3' (S8) and (ii) removing an aliquot of the first-stage reaction mixture and amplifying, using degenerate inner oligonucleotide primers in a second-stage polymerase chain reaction mixture, second targeted nucleotide sequences internal to the first

targeted nucleotide sequences, the second targeted nucleotide sequences comprising: (a) a 201 base-pair region of the polymerase gene from members of Vitivirus and Foveavirus genus; or (b) a 500-535 base-pair region of the HSP 70 homologue gene from members of Closterovirus genus, where the inner oligonucleotide primers specific for members of Vitivirus and Foveavirus genus comprise: Forward primer: 5'-GGGGCARACIHTIGCITGYTT-3' (S4), Reverse primers: 5'-AAIGCYTCRTARTCIGAITCNGT-3' (S5), where the inner oligonucleotide primers specific for members of Closterovirus genus comprise: Forward primers: 5'-TTYGGGACGACGTTYAGYAC-3' (S11) and 5'-TYGGGACGACGTTYTCNAC-3' (S12) Reverse primers: 5'-SCIGCIGMISWIGGYTCRTT-3' (S9) and 5'-GCGGMGSWGGG(dP)TCRTT-3' (S10); and (C) analyzing the first and second-stage PCR mixtures following amplification to detect the presence or absence of the first and second targeted nucleotide sequences, where the presence of the first and second targeted nucleotide sequences indicates the presence of the viruses in the sample suspected of being infected with the viruses. INDEPENDENT CLAIMS are included for the following: (1) oligonucleotide primers useful for detection of members belonging to Vitivirus, Foveavirus and Closterovirus genus, which are capable of specifically binding to: (i) species of Vitivirus, Foveavirus genus, and having a sequence of (S1)-(S5); and (ii) species of Closterovirus genus and having a sequence of (S6)-(S12); (2) kit for co-detecting members of Vitivirus, Foveavirus and Closterovirus genus in a sample, comprising: (a) oligonucleotide primers specific for Vitivirus and Foveavirus members comprising any of the sequences (S1)-(S5); and (b) oligonucleotide primers specific for Closterovirus members comprising any of the sequences (S6)-(S12); (3) kit for detecting members of Vitivirus, and Foveavirus genus in a sample, comprising oligonucleotide primers specific for Vitivirus and Foveavirus members comprising any of the sequences (S1)-(S5); and (4) kit for detecting members of Closterovirus genus in a sample, comprising oligonucleotide primers specific for Closterovirus members comprising any of the sequences (S6)-(S12).

BIOTECHNOLOGY - Preferred Method: The primers used are degenerate and contain deoxyinosine (dI). The dI-containing primers are used along with respective homologous degenerated primers, where dI is substituted by deoxyguanosine (dG) in regions of sequence homology. The degenerate inner primers used in the nested PCR amplification of homologus sequence regions of viti-, fovea-, and closteroviruses have a Tm at least 10degreesC higher than the outer oligonucleotide primers used in the first-stage **RT-PCR**.

USE - (M1) is useful for co-detecting members of Vitivirus, Foveavirus and Closterovirus genus in a sample suspected of being infected with the viruses, where the sample is tissue from species of Vitis (claimed).

ADVANTAGE - The method provides an optimized, highly sensitive, specific, reliable and inexpensive way of simultaneously detecting Vitivirus, Foveavirus, Closterovirus species in grapevine. The method involves an improved, simple sample preparation suitable for large scale applications. The method is simple, fast and cost-effective.

EXAMPLE - Total RNA was extracted from infected grapevine leaves according to a method described previously (Nassuth et al., 2000, J. Virol. Meth. 90, 37-49). Approximately 0.5 g tissue was homogenized with 5 ml lysis buffer (4 M guanidinium isothiocyanate, 0.2 M sodium acetate, pH 5, 25 mM EDTA, and 6% PVP-40 and 1% 2-mercaptoethanol). A 1 ml aliquot of the lysate was mixed with 67 microl 30% sarkosine and incubated for **10 minutes** at 70degreesC. Approximately 600 microl of the mixture was applied to a Qiashredder spin column and centrifuged for 2 minutes. Next, 250 microl of the mixture was transferred to a new **tube**, mixed with 225 microl of 95% ethanol, applied onto an Rneasy column and centrifuged. The column was washed with 700 microl RW1 and 500 microl RPE. An additional 500 microl RPE was loaded on the column and washed through by centrifugation for 5 minutes at 14000 g. Finally, the RNA was eluted from the column by applying 100 microl H2O, waiting for 1 minute and centrifuging for 60 seconds at 8000 g. A 25 microl

reaction volume was used, containing 1 microl of total RNA. The reaction mixture contained: 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X-100, 0.25 mM of each deoxyribonucleoside triphosphate (dNTP), 5 mM dithiothreitol (DTT), 5% dimethylsulfoxide (DMSO), 5 microg purified bovine serum albumin (BSA), 12 units RNASEOUT, 0.8 units Superscript (RTM) II Rnase H Reverse Transcriptase, 0.6 units Avian Myeloblastosis Virus Reverse Transcriptase, 0.8 units Dynazyme (RTM) II DNA polymerase, viti- and foveavirus specific primers (0.8 mM 5'-WGCIAARGCIGGICARAC-3'; 1 microM 5'-RMYTCICCISWRAAICKCAT-3'; and 0.8 microM 5'-GCCGSWRAAGCKCAT-3') and closterovirus specific primers (0.5 microM 5'-GGIHTIGAITTYGGIACIACITT-3'; 0.2 microM 5'-GTTYGGGACGACGTT-3'; and 1 microM 5'-GTICCICCICCNAARTC-3'). The cycling profile was as follows: first step at 42 degreesC for 50 minutes, second step at 50 degreesC for 1 minutes, third step at 94 degreesC for 4 minutes; five cycles segmented in step (a): 60 seconds at 95 degreesC, step (b): 10 seconds at 43degreesC, step (c): 5 seconds at 38 degreesC, step (d): 15 seconds at 72 degreesC; thirty five cycles segmented in step (a): 30 seconds at 95 degreesC, step (b): 30 seconds at 43 degreesC, step (c): 20 seconds at 72 degreesC, followed by a final extension step at 72 degreesC for 2 minutes. Twenty microl nested PCR reactions were performed using 1 microl directly from the first **RT-PCR** product. The reaction mixture contained 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X-100, 0.2 mM of each dNTP, 1 microg purified BSA, and 0.5 units Dynazyme (RTM) II DNA polymerase. For the detection of viti- and foveaviruses the following inner primers (1 microM 5'-GGGGCARACIHTIGCITGYTT-3' and 1.5 microM 5'-AAIGCYTCRTARTCIGAITCNGT-3') were used. The cycling profile consisted of a first denaturizing step at 95degreesC for 3 minutes, one step at 48degreesC for 15 seconds, one step at 72degreesC for 15 seconds and 39 cycles segmented in 20 seconds at 95degreesC, 30 seconds at 54degreesC, and 10 seconds at 72degreesC (+1 second after each cycle), followed by one final extension step at 72degreesC, for 2 minutes. For the detection of closteroviruses the same buffer composition and cycling profile were used along with the following inner primers (1 microM 5'-SCIGCIGMISWIGGYTCRTT-3'; 1 microM 5'-GCGGMGSWGGG(dP)TCRTT-3'; 5'-TTYGGGACGACGTTYAGYAC-3' and 5'-TYGGGACGACGTTYTCNAC-3'). The reaction products were analyzed by electrophoresis in 1.8% agarose gels in TAE buffer, stained with ethidium bromide, and visualized under UV light. (24 pages)

L5 ANSWER 7 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-15687 BIOTECHDS
TITLE: Quantifying nucleic acid e.g. mRNA amount in specimen, by quantitatively measuring nucleic acid amount in specimen, measuring cell type distribution in specimen, and correcting measured value of nucleic acid amount;
method for RNA quantification and DNA microarray or DNA chip analysis.
AUTHOR: SUDO Y; SOME M
PATENT ASSIGNEE: FUJI PHOTO FILM CO LTD
PATENT INFO: EP 1277842 22 Jan 2003
APPLICATION INFO: EP 2002-15665 17 Jul 2002
PRIORITY INFO: JP 2001-216568 17 Jul 2001; JP 2001-216568 17 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-373753 [36]
AN 2003-15687 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Quantifying (M) the amount of nucleic acid existing in a specimen, comprises quantitatively measuring the amount of nucleic acid in the specimen, measuring the distribution of cell types existing in the specimen, and correcting the measured value of the amount of nucleic acid based on the measured value of cell distribution.
BIOTECHNOLOGY - Preferred Method: The specimen comprises control

cells and target cells, the ratio of control cells to target cells in the specimen is assayed, and the amount of nucleic acid in the target cell is determined by correcting the measured value of the nucleic acid in the specimen using the cell ratio obtained and the amount of nucleic acid in the control cell. The control cell is a normal cell and the target cell is an abnormal cell. The method for measuring the nucleic acid in the specimen is the DNA array technique or **reverse transcriptase-polymerase chain reaction** (**RT-PCR**). The mRNA isolated from the specimen or a nucleic acid product synthesized from it is spotted on a chip having a probe nucleic acid immobilized on it, and the signal intensity of hybridization is detected to quantitatively measure the amount of nucleic acid in the specimen. The specimen is stained, cell nucleic acid are extracted by graphics extraction, the image characteristics of the extracted graphics is computed, and the cell type is determined based on the image characteristics so as to assay the ratio of control cells to target cells. The image characteristics are at least an area, boundary length and a complexity value, where complexity = area/(boundary length x boundary length). The specimen comprises control cells and target cells, and the amount of nucleic acid in the target cell is determined by correcting the measured value of the amount of nucleic acid in the specimen based on the following formula: (amount of nucleic acid in specimen - (amount of nucleic acid in control cell)xx)/y, where x represents the ratio of control cells in the specimen and y represents the ratio of target cells in the specimen. The specimen comprises a first control cell, a second control cell and target cells, and the amount of nucleic acid in the target cell is determined by correcting the measured value of the amount of nucleic acid in the specimen based on the following formula: (amount of nucleic acid in specimen - (amount of nucleic acid in first control cell)xx-(amount of nucleic acid in second control cell)xy)/z, where x represents a ratio of the first control cell in the specimen, y represents a ratio of the second control cell in the specimen, and z represents a ratio of the target cell in the specimen.

USE - (M) is useful for quantifying the amount of nucleic acid, preferably mRNA existing in a specimen. (M) is useful for diagnosing disease states by measuring the amount of mRNA being expressed in a specimen, by quantitatively measuring the amount of mRNA in the specimen, measuring the distribution of cell types existing in the specimen, and correcting the measured value of the mRNA based on the measured value of cell distribution. The measured value of mRNA in the specimen and the measured value of distribution of cell types existing in the specimen are simultaneously indicated (claimed). (M) is useful for measuring a gene in a target cell existing in a specimen (such as tissue slice) comprising two or more types of cells.

ADVANTAGE - (M) improves accuracy in the process for analyzing DNA or mRNA such as DNA microarrays or DNA chips. (M) improves the data reliability of a system for analyzing nucleic acids such as DNA microarrays or DNA chips.

EXAMPLE - Correction of mRNA expression data by the assay of cell distribution was as follows: An enzyme-labeled cDNA was first prepared. Human liver-derived cell lines (HEPG2) and/or human small intestine-derived cell lines (Caco-2) were mixed in such a way that the total number of cells were 100000. Total RNAs of sample 1 (100000 HEPG2 cells and no Caco-2 cells), sample 2 (no HEPG2 cells and 100000 Caco-2 cells) and sample 3 (400000 HEPG2 cells and 60000 Caco-2 cells) were mixed with 500 ng of a gene-specific primer (such as HSA1L: AAAGGAGTTCCGGGGCATAAAAG) mixture, and RNAse free sterilized water was added to bring the mixture to 12 micro litre. The mixture was heated at 70 degrees C for **10 minutes** and then quenched in ice bath. The content was collected at the bottom of the **tube** by a centrifuge, then 4 micro litre of 5 x first strand synthesizing buffer (250 mM Tris-HCl (pH 8.3), 375 mM KCl, and 15 mM MgCl2), 2 micro litres of 0.1 M dithiothreitol (DTT), 1 micro litre of mixture of 10 mM dATP,

dCTP and dGTP, 5 microl of 1 mM dUTP, and 5 micro litres of 1 mM fluorescein-dUTP were added and mixed, and the resultant mixture was incubated at 42 degrees C for 30 minutes, followed by heating at 70 degrees C for 15 minutes. Finally, 2 units of Escherichia coli-derived RNAse H were added and mixed, and the mixture was then incubated at 37 degrees C for 20 minutes. Serum albumin, alpha-2-HS glycoprotein, HFREP-1, E-cadherin, tetraspan NET-1, beta-actin, and an EF1-alpha gene fragment (about 500 bp) were prepared by polymerase chain reaction (PCR), purified and mixed. The mixture was heated at 95 degrees C and quenched. Subsequently, 1 micro litre of the product was spotted on a nylon membrane HyBond N+, air dried, and irradiated with UV. The DNA-spotted membrane was immersed in a hybridization buffer and shaken at 60 degrees C for 30 minutes. Thereafter, horseradish peroxidase (HRP)- and alkaline phosphatase (ALP)-labeled probes prepared were added to bring the final concentration to 5 ng/ml, and the mixture was further shaken at 60 degrees C for 16 hours. The above-described membrane was immersed in washing solution (1% SSC, 0.1% sodium dodecyl sulfate (SDS)) and shaken at 60 degrees C for **10 minutes**. This procedure was repeated three times. The membrane was rinsed once with buffer A (100 mM Tris-HCl (pH 7.5), 600 mM NaCl) and immersed in a solution which was prepared by diluting Liquid Block by 20-fold with the above buffer A, for 30 minutes. The membrane was then immersed in a solution which was prepared by diluting HRP-labeled anti-fluorescein antibody by 1000-fold with buffer A containing 0.5% bovine serum albumin (BSA), for 30 minutes, and then in 0.1% Tween 20 for **10 minutes** x 3 times.
This membrane was placed on a wrap film, and a sufficient amount of ECL detection reagent attached to the kit was added dropwise. 3 minutes later, the membrane was covered with a wrap, and light emission was detected and recorded. The measured value of sample 3 before correction (a mixture of human small intestine-derived cell lines and human liver-derived cell lines (4:6)) did not accurately indicate the mRNA expression pattern of human small intestine-derived cell lines. However, by correcting the value on as described above, it was clear that more accurate analysis of mRNA expression was carried out. This result demonstrated that correction of data on mRNA expression based on the ratio of existing cells led to more accurate analysis of mRNA expression. (21 pages)

L5 ANSWER 8 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-07325 BIOTECHDS
TITLE: Use of a nucleic acid encoding extracellular superoxide dismutase protein or an extracellular superoxide dismutase protein in the preparation of therapeutic composition for treating e.g. restenosis;
retro virus, Sendai virus, adeno-associated virus, adeno virus or liposome vector-mediated superoxide dismutase protein gene transfer and expression in host cell
AUTHOR: LAHTINEN M; LAUKANEN M; YLA-HERTTUALA S; LEPPAENEN O
PATENT ASSIGNEE: XENERATE AB
PATENT INFO: WO 2002087610 7 Nov 2002
APPLICATION INFO: WO 2002-SE848 30 Apr 2002
PRIORITY INFO: FI 2001-898 30 Apr 2001; FI 2001-898 30 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-093198 [08]
AN 2003-07325 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - In the preparation of a therapeutic composition to be administered systemically to a mammalian, a nucleic acid encoding extracellular superoxide dismutase (EC-SOD) protein and EC-SOD protein is used. Inhibition of hyperplastic connective tissue growth and/or promoting endothelialization in vivo at least partially on a synthetic surface implanted in the mammalian is enabled.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) A medical device with improved biological properties for an at least partial contact with blood, bodily fluids and/or tissues when introduced in a mammalian body, comprising: (a) a core; and (b) a nucleic acid in a biologically compatible medium. (b) encodes a translation or transcription product capable of leading to production of EC-SOD protein; or (c) an EC-SOD protein. The protein inhibits hyperplastic connective tissue or fibromuscular formation and/or promotes endothelialization in vivo at least partially on at least one synthetic surface of (a); (2) Use of the nucleic acid encoding EC-SOD and EC-SOD protein to improve the biological properties of a synthetic surface of the medical device. The nucleic acid and the protein in the biological compatible medium is contacted with the surface in solution or gel form; (3) Improving a mammalian body's biocompatibility with a synthetic surface involving introducing a device comprising at least one synthetic surface in a body with at least partial contact with blood, bodily fluids and/or tissues and administering (b) present in (c) to the surrounding. (b) is administered before, simultaneously or after the introduction of the device in the body; and (4) A method of producing the medical device involving providing the core comprising at least one surface of a synthetic material and providing (b) in the biological compatible medium.

ACTIVITY - Vasotropic; Anticoagulant.

MECHANISM OF ACTION - Hyperplastic connective tissue growth inhibitor; Endothelialization promoter. The effect of adenovirus mediated gene transfer of EC-SOD on intimal thickening was evaluated in a rabbit restenosis model. 28 New Zealand white rabbits were kept on 0.25% cholesterol rich diet for two weeks before balloon catheter mediated denudation of aortic endothelium. The animals were anaesthetized with subcutaneous (0.5 ml) Hypnorm (Janssen) and intramuscular (0.8 ml) Dormicum. Three days after the denudation EC-SOD or LacZ (control) adenovirus gene transfer (3x109 pfu/kg) was performed to the abdominal aortic segment. Serum samples were collected before the gene transfer, three and seven days after the gene transfer, and at the end of study. Two weeks or four weeks after the gene transfer the animals were sacrificed. Multiple tissue samples were collected to determine the biodistribution of adenovirus. Gene transfer site and adjacent segments of abdominal aorta from renal arteries to bifurcation point were analyzed histologically to determine the effect of adenoviral EC-SOD gene transfer on neointima formation. Aortic sections were obtained at 3 sites: the segment for gene transfer, a segment 2 cm proximal to, and a segment 2 cm distal from the gene transfer site. After removal of the **vessel** segments, the specimen were flushed gently with saline and divided into three equal parts. One part was immersion-fixed in 4% paraformaldehyde/15% sucrose (pH 7.4) for 4 hours, rinsed overnight in 15% sucrose (pH 7.4) and embedded in paraffin. Another part was fixed in 4% paraformaldehyde/15% sucrose (pH 7.4) for **10 minutes** , rinsed in PBS, embedded in OCT compound and stored at -70 degrees C until subsequent analysis of gene transfer efficiency by X-gal staining for 6 hours at +37 degrees C (LacZ transfected animals). The third part was snap-frozen and further stored at -70 degrees C until **RT-PCR** analysis (EC-SOD transfected animals). Neointima formation was quantified after hematoxylin/eosin staining. Antibody CD31 verified with vWF was used for signal detection and apoptosis was detected. SMC accumulation was a common consequence also after balloon angioplasty causing neointima formation within six months after the procedure as given in Bittl JA: Advances in coronary angioplasty N. Engl. J. Med. 1996; 335: 1290-1302. Aortic sections stained with CD31 for endothelial cells showed 86+/-13% recovery of **vessel** endothelium after denudation in EC-SOD group at two weeks time point whereas in LacZ control group the endothelial recovery was only 21+/-13% showing a significant difference (p is less than 0.001). Immunohistological analysis of factors which could be involved in this effect (eNOS, iNOS, VEGF-A, VEGF-C, and NF-kappaB) showed no difference between EC-SOD and

LacZ control groups. The endothelial recovery of the control samples reached EC-SOD group at four weeks time point being 88+/-13% for EC-SOD and 81+/-19% for LacZ control group.

USE - In the manufacture of a medicament for treating conditions (e.g. restenosis, blood **vessel** thickening) caused by damages due to vascular manipulations in a mammalian body (preferably human body) and for decreasing macrophage accumulation after vascular manipulation and in production of the medical device (e.g. cardiovascular implant, vascular graft, an endovascular implant, stent, stent graft, graft connector, a tissue plant, biosensor) (claimed).

ADMINISTRATION - (b) is administered in a naked form in a viral vector selected from retrovirus, Sendai virus, adeno associated virus or adenovirus or in a liposome. (b) is administered systemically to the mammalian or to the surroundings of the device before, simultaneously as or after the introduction of the device in the body. The medicament is administered locally or systemically (all claimed).

ADVANTAGE - The nucleic acid and the EC-SOD protein enables Inhibition of hyperplastic connective tissue growth and/or promoting endothelialization in vivo at least partially on a synthetic surface implanted in the mammalian. The device has improved biological properties for an at least partial contact with blood, bodily fluids and/or tissues when introduced in a mammalian body. The device improves a mammalian body's biocompatibility. The device is efficient with small-size synthetic **vessel** sections and intravascular implants that develop connective tissue hyperplasia. The composition enables inhibition of hyperplastic connective tissue growth and/or promotes endothelialization in vivo at least partially on a synthetic surface implanted in the mammalian.

EXAMPLE - No relevant example given. (85 pages)

L5 ANSWER 9 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-04720 BIOTECHDS
TITLE: Multiplex PCR method for detecting malignancies, e.g. adenocarcinoma of the esophagus comprises conducting a PCR amplification on a DNA sample in a PCR reaction mixture; polymerase chain reaction and DNA primer for disease diagnosis
AUTHOR: GODFREY T E; LUKETICH J D; RAJA S; KELLY L A; FINKELSTEIN S D
PATENT ASSIGNEE: UNIV PITTSBURGH
PATENT INFO: WO 2002070751 12 Sep 2002
APPLICATION INFO: WO 2002-US6504 4 Mar 2002
PRIORITY INFO: US 2001-273277 2 Mar 2001; US 2001-273277 2 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-732795 [79]
AN 2003-04720 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Multiplex polymerase chain reaction (PCR) (M1) comprising conducting PCR on a DNA sample in a reaction mixture conducted in first and second amplification stages, each with one or more PCR cycles comprising denaturing, annealing and elongating steps where the elongating step may be conducted at the same temperature as the annealing step, is new.

DETAILED DESCRIPTION - The second amplification stage of (M1) is conducted under different reaction conditions from that of the first amplification stage to modulate the relative rate of production of the first amplicon by a first primer set and a second amplicon by a second primer set during the first and second amplification stages. INDEPENDENT CLAIMS are also included for: (1) an oligonucleotide comprising 15-28 bp or its derivative; (2) intraoperative PCR diagnostic; (3) rapid detection of a malignancy or of metastasized adenocarcinoma of the esophagus; or (4) a **cartridge** for use in an automated PCR system.

BIOTECHNOLOGY - Preferred Method: The multiplex PCR further

comprises increasing sensitivity of a one-**tube RT-PCR** method by: (i) conducting a reverse transcription reaction on an RNA sample; (ii) adding a PCR reagent composition containing PCR primers and a thermostable DNA polymerase to the reaction mixture, to produce the DNA of the DNA sample of the reaction mixture; and (iii) conducting a PCR amplification on the reaction mixture. Prior to the PCR amplification, the PCR reagent composition is separated from the reaction mixture in a reaction **vessel** by a physical barrier, which is removed prior to or during the first cycle of the PCR reaction, so as to add the PCR reagent composition to the reaction mixture. The reverse transcription reaction is conducted for at most **10 minutes** or for 2 minutes. One or more reagents for the reaction mixture are provided for the reaction mixture in a **cartridge** suitably configured for an automated system. The automated system adds the reagents to a reaction **vessel** from the **cartridge** according to a programmed sequence. The concentration of each of the primers is at least about 400 nM. There is expected to be at least about a 30-100-fold difference in the number of target sequences of the first primer set and of the second primer set in the DNA sample. The first target sequence is expected to be at least about 30-fold more prevalent in the DNA sample than the second target sequence. One or both of the Internal Positive Control RNA and DNA are added to the reverse transcription reaction. An Internal Positive Control DNA, which contains one or more uracil residues, is added to the PCR reaction mixture and comprises the sequence consisting of 28-75 bp or its derivative. The amplification stages include quantitative PCR reactions using a fluorescent reporter to indicate accumulation of a specific amplicon. The quantitative PCR reactions are fluorescent 5' nuclease assays. The fluorescent reporter is a molecular beacon. The **cartridge** contains additional reagents or mechanical components compartmentalized separately or together with the reagents for the reaction mixture, the additional reagents or mechanical components adapted for one of cell or tissue lysis, RNA purification and reverse transcription. The automated system automatically shifts the PCR reaction from the first amplification stage to the second amplification stage when the fluorescent reporter accumulates in the reaction mixture to a threshold level. The first and second stages are conducted sequentially in the same reaction **vessel**. The second primer set is added to the reaction mixture at the beginning of the second amplification stage. The first and second PCR primer sets produce a beta-GUS-specific amplicon, an 18S RNA-specific amplicon, a CEA-specific amplicon or a tyrosinase-specific amplicon and includes a primer having a sequence comprising 15-23 bp or its derivative. The first primer set produces a B-GUS-specific amplicon and the second primer set produces a CEA-specific amplicon, the Tm of the first primer set being about 10degreesC lower than the Tm of the second primer set and the annealing temperature for the PCR amplification of the first amplification stage is about 10degreesC lower than the annealing temperature for the PCR amplification of the second amplification stage. The first PCR primer set consists of a sequence comprising 17 or 18 bp, and the second PCR primer set consists of 20 or 22 bp. The annealing step of the first amplification stage is conducted at a temperature greater or less than the annealing step of the second amplification stage. The annealing temperature for the PCR amplification of the first amplification stage is equivalent to about 53degreesC and the annealing temperature for the PCR amplification of the second amplification stage is equivalent to about 64degreesC, based on initial primer concentration of both PCR primer sets of 400 nM/L and an effective Tm for the first PCR primer set of about 50degreesC and an effective Tm for the second PCR primer set of about 60degreesC. The effective Tm of the first and second primer sets differ by at least about 5degreesC. The reaction mixture comprises a DNA sample, the first primer set having a first effective Tm and the second primer set having a second effective Tm different from the first effective Tm, where the annealing step of the first amplification

stage is conducted at a different temperature from the annealing step of the second amplification stage. The annealing and elongation steps in a PCR cycle of at least one of the first and second amplification stages are conducted at the same or different temperatures. The denaturation step for each cycle is at most 1 second. The annealing and elongation steps are less than about 10 seconds. Rapid detection of metastasized adenocarcinoma of the esophagus comprises: (I) obtaining RNA from a sentinel lymph node; (II) performing a quantitative **RT-PCR** method specific to CEA on the RNA; and (III) determining if expression of CEA exceeds a threshold level. The intraoperative PCR diagnostic method comprises: (a) obtaining a tissue sample from a patient in an operation; (b) analyzing the sample; (c) determining if expression of an indicator transcript exceeds a threshold level; and (d) continuing the operation in a manner dictated by results of the analyzing step. Rapid detection of a malignancy comprises: (i) obtaining nucleic acid from a tumor biopsy; (ii) performing a PCR method specific to an indicator transcript on the nucleic acid; and (iii) determining if expression of the indicator transcript exceeds a threshold level, which indicates a malignancy. The indicator transcript is CEA. Preferred **Cartridge**: The **cartridge** comprises one or more compartments containing a first PCR primer set and a second PCR primer set, the first PCR primer set and the second PCR primer set may be in the same compartment or in different compartments. The **cartridge**, which is disposable after a single use, has several compartments in which the reagents are stored prior to the use in **RT-PCR** method. It further comprises a third compartment including reverse transcription reagents, cell lysis reagents and RNA purification reagents.

USE - (M1) is useful for detecting malignancies, e.g. adenocarcinoma of the esophagus (claimed).

ADVANTAGE - (M1) eliminates contamination and decreases the time it takes to carry out a PCR reaction.

EXAMPLE - The 18S ribonucleic acid (RNA) (andbeta-Gus) endogenous control primers were redesigned so that the annealing temperature was 10degreesC below that of the CEA primers. The target gene primer concentrations were at 400 nM while those of the endogenous control were at 100 nM, the reverse transcriptase primer concentrations were at 60 nM each and the cycling conditions were modified as previously mentioned. In the CEA reactions, the singleplex reached threshold at 33.5 cycles, while the multiplexed CEA reached threshold at 34.5 cycles.(141 pages)

L5 ANSWER 10 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-00926 BIOTECHDS
TITLE: Early identification of germline transformed plants, by transforming meristematic tissue with nucleic acid encoding protein/antisense gene, producing shoot, growing root from shoot, assaying for nucleic acid in roots; transgenic plant construction via bacterium-mediated transformation for disease-resistance and yield enhancement
AUTHOR: MARTINELL B; JULSON L; BOURIAKOVA V; EMLER C; MCCABE D; PETERSEN M
PATENT ASSIGNEE: MONSANTO TECHNOLOGY LLC
PATENT INFO: WO 2002052025 4 Jul 2002
APPLICATION INFO: WO 2001-US49975 21 Dec 2001
PRIORITY INFO: US 2000-258137 22 Dec 2000; US 2000-258137 22 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-590603 [63]
AN 2003-00926 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Early identification (M) of germline transformed plants (TPs), involves transforming meristematic/cotyledonary tissue with plant

expressible construct having nucleic acid sequence (NA) encoding protein/antisense gene, to obtain TP tissue, producing shoot from tissue, growing roots from shoot, assaying roots for the presence of NA, and identifying roots that assay positive for NA as putative germline TPs.

DETAILED DESCRIPTION - Early identification (M) of germline transformed plants involves transforming meristematic or cotyledonary tissue with a plant expressible construct comprising a nucleic acid sequence encoding a protein or an antisense gene capable of affecting gene expression of an endogenous gene, to obtain transformed plant tissue, producing a shoot from the transformed plant tissue, growing roots from the shoot, assaying the roots for the presence of the nucleic acid sequence, and identifying roots that assay positive for the nucleic acid sequence as putative germline transformed plants. An INDEPENDENT CLAIM is also included for obtaining germline transformed plant cells by kanamycin selection involves providing a heterologous DNA construct comprising a promoter functional in plants operably connected to a DNA coding sequence, the coding sequence encoding a protein capable of conferring kanamycin resistance to a plant cell in which the sequence is expressed, and a 3 termination sequence, inserting the DNA construct into the meristematic tissue of a plant embryo, inducing shoot formation from the treated meristematic tissue by culturing the treated meristematic tissue on a culture medium, culturing the shoots on a suitable shooting medium containing kanamycin at a concentration sufficient to significantly inhibit the growth of untransformed plant cells to generate kanamycin resistant shoots, regenerating the shoots into genetically transformed plants having increased tolerance to kanamycin relative to wild-type plants, assaying the roots from the plants for the presence of the DNA construct, and identifying roots that assay positive for the DNA construct as putative germline transformed plants.

BIOTECHNOLOGY - Preferred Method: In (M), the plant expressible construct comprises a first nucleic acid sequence encoding a protein conferring a trait to the plant, the trait selected from genes that encode for insect tolerance, pest tolerance, herbicide resistance, quality enhancement, yield enhancement, stress tolerance, or environmental tolerance. The expressible construct comprises at least two nucleic acid sequences each encoding a protein. The first nucleic acid sequence encodes a protein conferring a trait to the plant and the second nucleic acid sequence encodes a protein permitting transformed plants to be selected in the presence of the corresponding selection agent. The roots are grown in the presence of a selection agent that corresponds to the protein. The selection agent is kanamycin or glyphosate. The transformation is through Agrobacterium-mediated or particle-mediated methods. The proteins is assayed by enzyme linked immunosorbent assay (ELISA), colorimetric methods, fluorometric methods, or by enzymatic methods. The nucleic acid is assayed by polymerase chain reaction (PCR), reverse transcriptase (**RT**)-**PCR** or by Southern blots.
The plant expressible construct comprises at least two nucleic acid sequences, the first encodes an antisense gene and the second encodes a protein permitting transformed plants to be selected in the presence of a corresponding selection agent. In M2, the DNA construct additionally comprises a gene of interest or nptII gene.

USE - (M) is useful for early identification of germline transformed plants (dicotyledonous plant e.g., soybean plant or cotton plant) (claimed), for the development of new selection agents, and for testing the efficiency of selection quickly and easily.

ADVANTAGE - (M) avoids the regeneration and propagation process wasted on the non-germline transformation events and thus assists in the efficient creation of lines of genetically transformed plants.

EXAMPLE - Soybeans were transformed by particle acceleration device and selected on either glyphosate or kanamycin. The glyphosate selection was done on plants that were transformed with 5'-enolpyruvyl-3'-phosposhikimate synthase (EPSPS) (a gene conferring tolerance to a glyphosate-containing herbicide), and kanamycin selection was done on

plants that were transformed with nptII. Embryonic axes were excised from seeds germinated in liquid bean germination medium (BGM) overnight at 20 degrees C in the dark. The primary leaf tissue was carefully removed to expose the meristematic region. The transformed plants were glyphosate selected as follows: Explants were plated on woody plant media (WPM) containing 2% sucrose, 200 mg/l carbenicillin, and 60 mg/l benomyl and incubated overnight at 15 degrees C, dark. The bead preparation for coating the blasting sheets was prepared as follows. One to five microl DNA (1 mg/ml) was added to 100 microl of 0.1 M spermidine. The spermidine/DNA solution was transferred to a **vessel** containing 10-20 mg of 0.82 or 0.95 microm beads and vortexed completely. One hundred microl 10% CaCl2 was added dropwise with continuous vortexing. The mixture was allowed to stand for **10 minutes**, during which time precipitation occurred. The supernatant was removed, and the DNA/gold precipitate was resuspended in 19 ml 100% ethanol plus 1 ml of sterile distilled water. A 320 microl aliquot of the bead preparation was used to coat each blasting sheet. For glyphosate selection, the DNA contained the gene for EPSPS synthase that confers resistance to glyphosate. Explants were transferred to target medium (8% low viscosity carboxymethylcellulose, 2% medium viscosity carboxymethylcellulose, 0.4% washed agar) with the meristems facing up. The explants were bombarded once, using an electric discharge particle mediated gene delivery instrument. Following bombardment, explants were transferred to WPM media plus 75 microM glyphosate. Shoots were cut 3-6 weeks post-bombardment and placed on BRM rooting media (undefined) containing 25-40 microM glyphosate. Shoots that did not root after 3 weeks were placed on BRM without selection for an additional 2-3 weeks. Root samples were taken from shoots that rooted off-selection. Root samples were taken from tissue culture and were assayed by CP4 dipstick enzyme linked immunosorbent assay (ELISA) or NPTII polymerase chain reaction (PCR). 75-100% correlation with plants rooting on glyphosate and germline transformation was found. Thus the above method is used for the early identification germline transformed plants. (18 pages)

L5 ANSWER 11 OF 13 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:234254 BIOSIS
DOCUMENT NUMBER: PREV200300234254
TITLE: Functions of rat hepatocytes under three-dimensional culture and simulated microgravity.
AUTHOR(S): Jiang Qing-Yan [Reprint Author]; Zhang Shou-Quan [Reprint Author]; Lao Quan-Lin [Reprint Author]; Gao Shu-Jing [Reprint Author]; Fu Wei-Long [Reprint Author]
CORPORATE SOURCE: College of Animal Science, South China Agricultural University, Guangzhou, 510642, China
qyjiang@scau.edu.cn
SOURCE: Acta Zoologica Sinica, (December 2002) Vol. 48, No. 6, pp. 770-776. print.
CODEN: TWHPA3. ISSN: 0001-7302.
DOCUMENT TYPE: Article
LANGUAGE: Chinese
ENTRY DATE: Entered STN: 14 May 2003
Last Updated on STN: 14 May 2003

AB 30-day-old Sprague-Dawley rats were selected for the isolation of hepatocytes by the method of in situ perfusion. A needle was inserted into the foramen occipital magnum of each rats and cerebral paralysis was induced by brain lesion. The abdominal cavity was opened to expose the liver. Collagenase (type I) at the concentration of 0.5 g/L was continuously perfused into the postcaval vein for **10 minutes**. The liver was removed and placed in a Petri dish and hepatocytes released after the capsula were torn to pieces with forceps. The hepatocytes were collected and washed three times with RPMI 1640 medium. In order to get scaffolds for hepatocyte culture, blood from

adult rats was collected by removing their eyeball and placing these in a Petri dish. After coagulation, resultant blood clots were rinsed thoroughly with 0.9% NaCl. The fiblin from the blood was lyophilized and cut into 1 mm3 pieces. After sterilization by exposure to 60Co, the fiblin was stored at 4degreeC. To create a monolayer culture, the fiblin was spread over six-well plates, and the medium of RPMI 1640 supplemented with 15% fetal sera was added into the plates. Hepatocytes were inoculated onto the fiblin at a concentration of 2X105 cells/ml. The plates were then put into the incubator and cultured at 37degreeC and the hepatocytes observed daily under a microscope. On days 2, 3, 5, 7, 9, 11, 14 and 18 of culture, the medium was collected and the concentrations of albumin and bile acid in medium it tested with an Automatic Chemical Analyzer (Beckman CX-7). In the three-dimension culture, the simulated microgravity condition was created with a Rotating-wall **Vessel** Bioreactor (RWVB). After the hepatocytes and the fiblin were mixed and pre-cultured in RPMI 1640 for 30 minutes, the mixture was transferred into RWVB with the final concentration of hepatocytes being 2X105 cells/ml. The rotating speed of the **vessel** was set at 15 rpm in the first week of culture, 20 rpm in the second week and 30 rpm for the final stage. The hepatocytes were observed with a microscope. On days 2, 3, 5, 7, 9, 11, 14, 18, 20, 24 and, 28 of culture, the medium was collected and the concentrations of albumin and bile acid in medium tested in the same way as for the monolayer culture. To compare the transcript expression of the genes of glucose 6-phosphate dehydrogenase (G6PDH), phospho-fructokinase (PFK), and phosphoglucomutase (PGM) of hepatocytes cultured in both the monolayer and three-dimensional condition, RNA was extracted from hepatocytes cultured on day 2, 4, and 6 in the monolayer condition and from hepatocytes cultured on day 28 in the three-dimension condition. RNA from hepatocytes isolated from fresh liver tissue was also extracted as a control. The mRNA of G6PDH, PFK and PGM in all the above samples was detected by nested **RT-PCR**. The results showed that rat hepatocytes cultured under simulated microgravity conditions began to attach to the scaffolds in RWVB after 12 hours of inoculation. Forty-eight hours after inoculation, most of the cells had aggregated on the edge of scaffolds and small clumps of cells had emerged. After one week of inoculation, the hepatocytes clumps were connected with each other through an extra-cellular matrix which made the clumps grow larger. After 28 days of culture, hepatocytes clumps with diameters of 500 to 600 micrometers each containing 200 to 300 cells had formed. Compared to hepatocytes cultured under monolayer conditions, which were markedly stretched and deformed, hepatocytes cultured under simulated microgravity remained spherical throughout the entire period of 28 days of culture. Moreover, hepatocytes cultured under simulated microgravity could continuously secrete albumin and bile acid throughout all 28 days of culture, while those under monolayer conditions could do so only within the first 18 days of culture. The transcript expression of the genes of glucose 6-phosphate dehydrogenase (G6PDH), phospho-fructokinase (PFK), and phosphoglucomutase (PGM) could be detected by nested **RT-PCR** in microgravity-cultured hepatocytes after 28 days of culture. In contrast, the expression of the genes of PFK and PGM genes could not be detected in monolayer-cultured hepatocytes after just 6 days of culture. These observations indicated that hepatocytes cultured under simulated microgravity could maintain not only their spherical shape, but also the functions of secretion and glucose metabolism, while the monolayer-cultured hepatocytes showed a gradual decline of secretive functions and abnormal glucose metabolism.

L5 ANSWER 12 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2001379114 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11435793
TITLE: Induction of the HSP110/105 family in the rat hippocampus in cerebral ischemia and ischemic tolerance.
AUTHOR: Yagita Y; Kitagawa K; Ohtsuki T; Tanaka S; Hori M;

Matsumoto M
CORPORATE SOURCE: Division of Strokology, Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, Osaka, Japan.
SOURCE: Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism, (2001 Jul) 21 (7) 811-9.
Journal code: 8112566. ISSN: 0271-678X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010730
Last Updated on STN: 20010730
Entered Medline: 20010726

AB Recently, the authors isolated a novel gene of the HSP110 family, ischemia responsive protein 94 kDa (irp94), and demonstrated the expression of this gene after transient forebrain ischemia. In the current study, the authors investigated the expression profiles of all HSP110 family members including hsp110/105 and osp94/apg-1, after transient forebrain ischemia using rat four-**vessel** occlusion model. Among three members of the HSP110 family, induction of hsp110/105 was the most prominent after ischemia. hsp110/105 mRNA expression was clearly enhanced from 4 to 24 hours after a 6-minute or longer ischemic period. First, hsp110/105 mRNA expression was induced in the dentate gyrus, and later in the pyramidal layer. HSP110/105 protein expression also was enhanced by a 6-minute or longer period of ischemia. Profiles of HSP110/105 expression after ischemia were similar to those of inducible HSP70. After transient forebrain ischemia for **10 minutes**, HSP110/105 protein was induced in the dentate gyrus and the CA3 pyramidal layer, but not in the CA1 pyramidal neurons. However, 6 minutes of ischemia induced the HSP110/105 protein, as well as the HSP70 protein, in the CA1 region. CA1 pyramidal neurons expressing HSP110/105 acquired tolerance against subsequent severe ischemia. In conclusion, HSP110/105 showed the most prominent induction after ischemia among the three HSP110 gene family members. Colocalization of HSP110/105 and HSP70 in the CA1 neurons that acquired tolerance suggested that induced HSP110/105 might contribute to ischemic tolerance together with HSP70.

L5 ANSWER 13 OF 13 MEDLINE on STN
ACCESSION NUMBER: 2000062921 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10596012
TITLE: Autoclaving eliminates hepatitis C virus from a hemodialysis monitor contaminated artificially.
AUTHOR: Barril G; Bartolome J; Traver J A; Cabrerizo M; Selgas R; Carreno V
CORPORATE SOURCE: Department of Nephrology, Hospital Universitario de la Princesa, Madrid, Spain.
SOURCE: Journal of medical virology, (2000 Feb) 60 (2) 139-43.
Journal code: 7705876. ISSN: 0146-6615.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000204
Last Updated on STN: 20000204
Entered Medline: 20000124

AB Nosocomial transmission of the hepatitis C virus (HCV) has become the principal cause of HCV infection in hemodialysis units. Because HCV particles may pass through dialysis membranes and backfiltration occurs with high performance membranes, HCV transmission from contaminated

dialysis monitors is likely. Thus it is important to have effective measures to disinfect hemodialysis monitors. In this study, autoclaving dialysate circuits were examined to establish an effective method to eliminate HCV particles from a monitor contaminated artificially. The dialysis monitor was contaminated in 2 different experiments with a 1/10 and 1/5 dilution of a serum pool containing 1.2 +/- 0.3 x 10(6) HCV genome copies/ml. During perfusion 2 samples were taken from the drainage **tube** at 5 and **10 minutes**. After perfusion, the dialysate circuit was autoclaved at 120 degrees C for 20 minutes. Four samples were then taken from the autoclaved circuits and another from the drainage, which had not been autoclaved. The viral titer in the samples from the drainage before sterilization was similar to that of the serum dilution, showing the homogeneous distribution of the serum dilution in the dialysis circuits. After autoclaving, HCV RNA was not detectable in the samples obtained from the autoclaved circuits, whereas it was positive in the sample from the drainage. These results show that autoclaving is an effective method to eliminate HCV particles from contaminated hemodialysis monitors.


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=> D HIS

     (FILE 'HOME' ENTERED AT 10:32:06 ON 19 JUL 2005)

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 10:32:18 ON 19 JUL 2005

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 10:32:54 ON 19 JUL 2005
L1       301500 S (RT-PCR OR REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION)
L2            1 S L1 AND (LESS (5A) 10 MINUTE###)
L3          153 S L1 AND 10 MINUT###
L4           13 S L3 AND (SINGLE TUBE OR TUBE OR VESSEL OR CARTRIDGE)
L5           13 DUP REM L4 (0 DUPLICATES REMOVED)

=> S ONE-STEP RT-PCR
L6          478 ONE-STEP RT-PCR

=> DUP REM L6
PROCESSING COMPLETED FOR L6
L7          197 DUP REM L6 (281 DUPLICATES REMOVED)

=> S L7 AND (10 MINUTES)
L8            3 L7 AND (10 MINUTES)

=> D IBIB ABS L8 1-3

L8    ANSWER 1 OF 3  BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-09913  BIOTECHDS
TITLE:            Nucleic acids encoding secreted polypeptides, designated
                  GPCRX polypeptides, useful for treating a GPCRX-associated
                  disorder, e.g. cardiomyopathy, atherosclerosis, diabetes and
                  metabolic disorders;
                     vector-mediated G-protein coupled receptor gene transfer,
                     expression in host cell and antibody for recombinant
                     protein production, drug screening and gene therapy
AUTHOR:           ZERHUSEN B; PADIGARU M; LI L; BURGESS C E; CASMAN S J; SPYTEK
                  K A; MISHRA V; TAYLOR S; SHENOY S; VERNET C; GERLACH V;
                  ELLERMAN K; MACDOUGALL J R; STONE D; SMITHSON G; GROSSE W M;
                  ALSOBROOK J P; LEPLEY D M; TCHERNEV V T; TAILLON B
PATENT ASSIGNEE:  CURAGEN CORP
PATENT INFO:      WO 2002006345 24 Jan 2002
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APPLICATION INFO: WO 2000-US22637 18 Jul 2000
PRIORITY INFO: US 2001-278917 26 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-171802 [22]
AN 2002-09913 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Isolated nucleic acids encoding novel G-protein coupled receptor polypeptides, designated GPCRX polypeptides (i.e. a GPCR polypeptide where X is an integer from 1 to 9), are new.

DETAILED DESCRIPTION - Isolated nucleic acids encoding novel G-protein coupled receptor polypeptides, designated GPCRX polypeptides (i.e. a GPCR polypeptide where X is an integer from 1 to 9), are new. A nucleic acid (N1) encoding a GPCRX polypeptide is selected from: (a) a nucleic acid encoding a mature form of a polypeptide selected from one of the 19 amino acid sequences (P1) defined in the specification; (b) a nucleic acid encoding a variant of a mature form of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the sequence of the mature form; (c) a nucleic acid encoding an amino acid sequence selected from P1; (d) a nucleic acid encoding a variant of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the amino acid sequence selected from P1; (e) a nucleic acid fragment encoding at least a portion of an amino acid sequence selected from P1, or a variant of the polypeptide, where one or more residues in the variant differs from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the amino acid sequence selected from P1; or (f) a nucleic acid comprising the complement of (a)-(e). INDEPENDENT CLAIMS are also included for the following: (1) an isolated polypeptide (P2) comprising an amino acid sequence selected from: (a) a mature form of a polypeptide selected from P1; (b) a variant of a mature form of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the sequence of the mature form; (c) an amino acid sequence selected from P1; (d) a variant of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the amino acid sequence selected from P1; (2) a vector comprising N1 operably linked to a promoter; (3) a cell comprising the vector of (2); (4) an antibody (Ab1) that immunospecifically binds to P2; (5) a method of determining the presence or amount of P2 in a sample, comprising contacting the sample with Ab1, and determining the presence or amount of Ab1 bound to the polypeptide, therefore determining the presence or amount of P2 in the sample; (6) a method (M1) of determining the presence or amount of N1 in a sample, comprising contacting the sample with a probe that binds to N1, and determining the presence or amount of the probe bound to the nucleic acid; (7) a method (M2) of identifying an agent that binds to P2, comprising contacting the polypeptide with the agent and determining whether the agent binds to the polypeptide; (8) a method of identifying an agent that modulates the expression or activity of P2; (9) a method for modulating the activity of P2, comprising contacting a cell sample expressing P2 with a compound that binds to the polypeptide in an amount sufficient to modulate the activity of the polypeptide; (10) a method of treating or preventing a GPCRX-associated disorder in a human, comprising administering P2, N1 or Ab1; (11) a kit comprising P2, N1 or Ab1; (12) a method for determining the presence of or predisposition to a disease, preferably cancer, associated with altered levels of P2 in a first mammalian subject; (13) a method for determining the presence of or predisposition to a disease, preferably cancer, associated with altered levels of N1 in a first mammalian

subject; (14) a method of treating a pathological state in a mammal, comprising administering a polypeptide in an amount that is sufficient to alleviate the pathological state, where the polypeptide has a sequence at least 95% identical to a polypeptide comprising an amino acid sequence selected from P1, or its biologically active fragment; (15) a method of treating a pathological state in a mammal, comprising administering Ab1 in an amount that is sufficient to alleviate the pathological state; (16) a method for the screening of a candidate substance interacting with an olfactory receptor polypeptide selected from P1, or their fragments or variants; (17) a method for screening of ligand molecules interacting with an olfactory receptor polypeptide selected from P1; and (18) a method for screening of ligand molecules interacting with an olfactory receptor polypeptide selected from P1.

WIDER DISCLOSURE - Also disclosed as new are: (1) antisense nucleic acid molecules hybridizable to or complementary to a sequence selected from one of the 12 nucleotide sequences defined in the specification; (2) fusion proteins comprising a GPCRX polypeptide; and (3) a method for monitoring the effectiveness of treatment of a subject with an agent e.g. antagonist, agonist, polypeptide, etc.

BIOTECHNOLOGY - Preferred Polypeptide: P2, preferably, comprises a sequence of a naturally occurring allelic variant of a sequence selected from P1. The allelic variant comprises a sequence that is a translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from one of the 19 nucleotide sequences (N2) defined in the specification. The amino acid sequence of the variant comprises a conservative substitution. Preferred Nucleic Acid: N1 comprises a nucleotide sequence of a naturally occurring allelic variant. N1 encodes a polypeptide comprising an amino acid sequence of a naturally occurring variant. The nucleic acid differs by a single nucleotide from a sequence selected from N2. Preferably, N1 is selected from: (a) a nucleotide sequence selected from N2; (b) a nucleotide sequence differing by one or more nucleotides from a nucleotide sequence selected from N2, provided that no more than 20% of the nucleotides differ from the sequence; (c) a nucleic acid fragment of (a) or (b); (d) a nucleotide sequence comprising a coding sequence (C1) differing by one or more nucleotide sequences from a coding sequence (C2) encoding an amino acid sequence selected from P1, provided that no more than 20% of the nucleotides in C1 differ from the nucleotides in C2; (e) an isolated polynucleotide that is a complement of the nucleotide of (d); (f) a nucleic acid fragment of (d) or (e). N1 hybridizes under stringent conditions to a sequence selected from N2 or its complementary sequence. Preferred Antibody: The antibody is humanized or monoclonal. Preferred Method: In M1, the presence or amount of the nucleic acid is used as a marker for cell or tissue type. The cell or tissue type is cancerous. In M2, the agent is a cellular receptor or a downstream effector. The method of (9) comprises: (a) providing a cell expressing the polypeptide; (b) contacting the cell with the agent; and (c) determining whether the agent modulates expression or activity of the polypeptide, where an alteration in expression or activity of the polypeptide indicates that the agent modulates expression or activity of the polypeptide. The method of (12) comprises: (a) measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and (b) comparing the amount of the polypeptide in the sample of step (a) to the amount of polypeptide present in a control sample from a second mammalian subject known not to have, or not to be predisposed to the disease, where an alteration in the expression level of the polypeptide in the first subject as compared to the control sample indicates the presence of or predisposition to the disease. The method of (13) comprises: (a) measuring the amount of the nucleic acid in a sample from the first mammalian subject; and (b) comparing the amount of the nucleic acid in the sample of step (a) to the amount of the nucleic acid present in a control sample from a second mammalian subject known not to have, or not to be predisposed to the disease, where an alteration in the expression level of the nucleic acid

in the first subject as compared to the control sample indicates the presence of or predisposition to the disease. The method of (16) comprises: (a) providing a polypeptide selected from P1, or its peptide fragment or a variant; (b) obtaining a candidate substance; (c) bringing into contact the polypeptide with the candidate substance; and (d) detecting the complexes formed between the polypeptide and the candidate substance. The method of (17) comprises: (a) providing a recombinant eukaryotic host cell containing a nucleic acid encoding a polypeptide selected from P1; (b) preparing membrane extracts of the recombinant eukaryotic host cell; (c) bringing into contact the membrane extracts prepared at step (b) with a selected ligand molecule; and (d) detecting the production level of second messengers metabolites. The method of (18) comprises: (a) providing an adenovirus containing a nucleic acid encoding a polypeptide selected from P1; (b) infecting an olfactory epithelium with the adenovirus; (c) bringing into contact the olfactory epithelium (b) with a selected ligand molecule; and (d) detecting the increase of the response to the ligand molecule. Isolation: The GPCR polynucleotides are isolated using standard genetic engineering techniques.

ACTIVITY - Cardiant; Antidiabetic; antiarteriosclerotic; antibacterial; antifungal; antiviral; antiprotozoal; analgesic; cytostatic; anoretic; appetite stimulant; Antiparkinsonian; nootropic; neuroprotective; antidepressant; anxiolytic; immunomodulatory. No biological data given.

MECHANISM OF ACTION - Gene therapy; GPCRX antagonist; GPCRX agonist. No biological data given.

USE - The GPCRX polypeptide, nucleic acid and antibody are useful for treating or preventing a GPCRX-associated disorder in humans. For example, the polypeptide and nucleic acid are useful for treating or preventing cardiomyopathy, atherosclerosis and disorders related to cell signal processing and metabolic pathway modulation. The antibody is useful for treating or preventing diabetes and disorders related to cell signal processing and metabolic pathway modulation (all claimed). The GPCRX polypeptide, nucleic acid and antibody are useful for the treatment or diagnosis of other GPCRX-associated disorders, e.g. bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease, multiple sclerosis, angina pectoris, myocardial infarction, ulcers, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders, the metabolic syndrome X, wasting disorders associated with chronic diseases, metabolic disorders, obesity, infectious disease, cancer-associated cachexia, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

ADMINISTRATION - The GPCRX nucleic acids, polypeptides and antibodies are administered parenterally, e.g. intravenous, intradermal, subcutaneous, oral, transdermal, transmucosal or rectal administration. No specific dosages are given in the specification.

EXAMPLE - The quantitative expression of various clones was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative polymerase chain reaction (PCR) (RTQ PCR; TAQMAN (RTM)). RTQ PCR was performed on a Perkin-Elmer Biosystems ABI PRISM 7700 Sequence Detection System. Various collections of samples are assembled on the plates, and referred to as Panel 1 (containing cells and cell lines from normal and cancer sources), Panel 2 (containing samples derived from tissues, in particular from surgical samples, from normal and cancer sources), Panel 3 (containing samples derived from a wide variety of cancer sources), Panel 4 (containing cells and cell lines from normal

cells and cells related to inflammatory conditions) and Panel CNSD.01 (containing samples from normal and diseased brains).First, the RNA samples were normalized to constitutively expressed genes such as beta--actin and GAPDH. RNA (approximately 50 ng total or approximately 1 ng polyA+) was converted to cDNA using the TAQMAN (RTM) Reverse Transcription Reagents Kit and random hexamers according to the manufacturer's protocol. Reactions were performed in 20 microlitres and incubated for 30 minutes at 48 degrees Centigrade. cDNA (5 microlitres) was then transferred to a separate plate for the TAQMAN (RTM) reaction using beta-actin and GAPDH TAQMAN (RTM) Assay Reagents and TAQMAN (RTM) universal PCR Master Mix according to the manufacturer's protocol. Reactions were performed in 25 microlitres using the following parameters: 2 minutes at 50 degrees Centigrade; **10 minutes** at 95 degrees Centigrade; 15 seconds at 95 degrees Centigrade/1 minute at 60 degrees Centigrade (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for beta-actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their beta-actin /GAPDH average CT values. Normalized RNA (5 microlitres) was converted to cDNA and analyzed via TAQMAN (RTM) using **One Step RT-PCR** Master Mix Reagents and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's Primer Express Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration 250 nM, primer melting temperature (Tm) range = 58-60 degrees Centigrade, primer optimal Tm = 59 degrees Centigrade, maximum primer difference = 2 degrees Centigrade, probe does not have 5' G, probe Tm must be 10 degrees Centigrade greater than primer Tm, amplicon size 75 base pairs (bp) to 100 bp. The probes and primers selected were synthesized by Synthegen. Probes were double purified by high pressure liquid chromatography (HPLC) to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM. Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate. PCR cocktails including two probes (a probe specific for the target clone and another gene-specific probe multiplexed with the target probe) were set up using 1 x TAQMAN (TM) PCR Master Mix for the PE Biosystems 7700, with 5 mM magnesium chloride, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold (TM), and 0.4 U/ml RNase inhibitor, and 0.25 U/ml reverse transcriptase. Reverse transcription was performed at 48 degrees Centigrade for 30 minutes followed by amplification/PCR cycles as follows: 95 degrees Centigrade for **10 minutes**, then 40 cycles of 95 degrees Centigrade for 15 seconds, 60 degrees Centigrade for 1 minute. The results from each panel could then be analyzed.(213 pages)

L8 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-09612 BIOTECHDS
TITLE: Nucleic acids encoding secreted polypeptides, designated GPCRX polypeptides, useful for treating a GPCRX-associated disorder, e.g. cardiomyopathy, atherosclerosis, diabetes and metabolic disorders;
vector-mediated G-protein coupled receptor gene transfer,

expression in host cell for recombinant protein production, drug screening and gene therapy

AUTHOR: PADIGARU M; MISHRA V; PATTURAJUAN M; TAILLON B; CASMAN S J; WOLENC A R; LI L; KEKUDA R; SPYTEK K A
PATENT ASSIGNEE: CURAGEN CORP
PATENT INFO: WO 2002006342 24 Jan 2002
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DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-164782 [21]
AN 2002-09612 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Isolated nucleic acids encoding novel G-protein coupled receptor polypeptides, designated GPCRX polypeptides (i.e. a GPCR polypeptide where X is an integer from 1 to 8), are new.

DETAILED DESCRIPTION - Isolated nucleic acids encoding novel G-protein coupled receptor polypeptides, designated GPCRX polypeptides (i.e. a GPCR polypeptide where X is an integer from 1 to 8), are new. A nucleic acid (N1) encoding a GPCRX polypeptide is selected from: (a) a nucleic acid encoding a mature form of a polypeptide selected from one of the 13 amino acid sequences (P1) defined in the specification; (b) a nucleic acid encoding a variant of a mature form of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the sequence of the mature form; (c) a nucleic acid encoding an amino acid sequence selected from P1; (d) a nucleic acid encoding a variant of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the amino acid sequence selected from P1; (e) a nucleic acid fragment encoding at least a portion of an amino acid sequence selected from P1, or a variant of the polypeptide, where one or more residues in the variant differs from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the amino acid sequence selected from P1; or (f) a nucleic acid comprising the complement of (a)-(e). INDEPENDENT CLAIMS are also included for the following: (1) an isolated polypeptide (P2) comprising an amino acid sequence selected from: (a) a mature form of a polypeptide selected from P1; (b) a variant of a mature form of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the sequence of the mature form; (c) an amino acid sequence selected from P1; (d) a variant of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the amino acid sequence selected from P1; (2) a vector comprising N1 operably linked to a promoter; (3) a cell comprising the vector of (2); (4) an antibody (Ab1) that immunospecifically binds to P2; (5) a method of determining the presence or amount of P2 in a sample, comprising contacting the sample with Ab1, and determining the presence or amount of Ab1 bound to the polypeptide, therefore determining the presence or amount of P2 in the sample; (6) a method (M1) of determining the presence or amount of N1 in a sample, comprising contacting the sample with a probe that binds to N1, and determining the presence or amount of the probe bound to the nucleic acid; (7) a method (M2) of identifying an agent that binds to P2, comprising contacting the polypeptide with the agent and determining whether the agent binds to the polypeptide; (8) a method of identifying an agent that modulates the expression or activity of P2; (9) a method for modulating the activity of P2, comprising contacting a cell sample expressing P2 with a compound that binds to the polypeptide in an amount sufficient to modulate the activity of the polypeptide; (10) a method of treating or preventing a GPCRX-associated

disorder in a human, comprising administering P2, N1 or Ab1; (11) a kit comprising P2, N1 or Ab1; (12) a method for determining the presence of or predisposition to a disease, preferably cancer, associated with altered levels of P2 in a first mammalian subject; (13) a method for determining the presence of or predisposition to a disease, preferably cancer, associated with altered levels of N1 in a first mammalian subject; (14) a method of treating a pathological state in a mammal, comprising administering a polypeptide in an amount that is sufficient to alleviate the pathological state, where the polypeptide has a sequence at least 95% identical to a polypeptide comprising an amino acid sequence selected from P1, or its biologically active fragment; (15) a method of treating a pathological state in a mammal, comprising administering Ab1 in an amount that is sufficient to alleviate the pathological state; (16) a method for the screening of a candidate substance interacting with an olfactory receptor polypeptide selected from P1, or their fragments or variants; (17) a method for screening of ligand molecules interacting with an olfactory receptor polypeptide selected from P1; and (18) a method for screening of ligand molecules interacting with an olfactory receptor polypeptide selected from P1.

WIDER DISCLOSURE - Also disclosed as new are: (1) antisense nucleic acid molecules hybridizable to or complementary to a sequence selected from one of the 12 nucleotide sequences defined in the specification; (2) fusion proteins comprising a GPCRX polypeptide; and (3) a method for monitoring the effectiveness of treatment of a subject with an agent e.g. antagonist, agonist, polypeptide, etc.

BIOTECHNOLOGY - Preferred Polypeptide: P2, preferably, comprises a sequence of a naturally occurring allelic variant of a sequence selected from P1. The allelic variant comprises a sequence that is a translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from one of the 13 nucleotide sequences (N2) defined in the specification. The amino acid sequence of the variant comprises a conservative substitution. Preferred Nucleic Acid: N1 comprises a nucleotide sequence of a naturally occurring allelic variant. N1 encodes a polypeptide comprising an amino acid sequence of a naturally occurring variant. The nucleic acid differs by a single nucleotide from a sequence selected from N2. Preferably, N1 is selected from: (a) a nucleotide sequence selected from N2; (b) a nucleotide sequence differing by one or more nucleotides from a nucleotide sequence selected from N2, provided that no more than 20% of the nucleotides differ from the sequence; (c) a nucleic acid fragment of (a) or (b); (d) a nucleotide sequence comprising a coding sequence (C1) differing by one or more nucleotide sequences from a coding sequence (C2) encoding an amino acid sequence selected from P1, provided that no more than 20% of the nucleotides in C1 differ from the nucleotides in C2; (e) an isolated polynucleotide that is a complement of the nucleotide of (d); (f) a nucleic acid fragment of (d) or (e). N1 hybridizes under stringent conditions to a sequence selected from N2 or its complementary sequence. Preferred Antibody: The antibody is humanized or monoclonal. Preferred Method: In M1, the presence or amount of the nucleic acid is used as a marker for cell or tissue type. The cell or tissue type is cancerous. In M2, the agent is a cellular receptor or a downstream effector. The method of (8) comprises: (a) providing a cell expressing the polypeptide; (b) contacting the cell with the agent; and (c) determining whether the agent modulates expression or activity of the polypeptide, where an alteration in expression or activity of the polypeptide indicates that the agent modulates expression or activity of the polypeptide. The method of (12) comprises: (a) measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and (b) comparing the amount of the polypeptide in the sample of step (a) to the amount of polypeptide present in a control sample from a second mammalian subject known not to have, or not to be predisposed to the disease, where an alteration in the expression level of the polypeptide in the first subject as compared to the control sample indicates the presence of or predisposition to the

disease. The method of (13) comprises: (a) measuring the amount of the nucleic acid in a sample from the first mammalian subject; and (b) comparing the amount of the nucleic acid in the sample of step (a) to the amount of the nucleic acid present in a control sample from a second mammalian subject known not to have, or not to be predisposed to the disease, where an alteration in the expression level of the nucleic acid in the first subject as compared to the control sample indicates the presence of or predisposition to the disease. The method of (16) comprises: (a) providing a polypeptide selected from P1, or its peptide fragment or a variant; (b) obtaining a candidate substance; (c) bringing into contact the polypeptide with the candidate substance; and (d) detecting the complexes formed between the polypeptide and the candidate substance. The method of (17) comprises: (a) providing a recombinant eukaryotic host cell containing a nucleic acid encoding a polypeptide selected from P1; (b) preparing membrane extracts of the recombinant eukaryotic host cell; (c) bringing into contact the membrane extracts prepared at step (b) with a selected ligand molecule; and (d) detecting the production level of second messengers metabolites. The method of (18) comprises: (a) providing an adenovirus containing a nucleic acid encoding a polypeptide selected from P1; (b) infecting an olfactory epithelium with the adenovirus; (c) bringing into contact the olfactory epithelium (b) with a selected ligand molecule; and (d) detecting the increase of the response to the ligand molecule. Isolation: The GPCR polynucleotides are isolated using standard genetic engineering techniques.

ACTIVITY - Cardiant; Antidiabetic; antiarteriosclerotic; antibacterial; antifungal; antiviral; antiprotozoal; analgesic; cytostatic; anoretic; appetite stimulant; Antiparkinsonian; nootropic; neuroprotective; antidepressant; anxiolytic; immunomodulatory. No biological data given.

MECHANISM OF ACTION - Gene therapy; GPCRX antagonist; GPCRX agonist. No biological data given.

USE - The GPCRX polypeptide, nucleic acid and antibody are useful for treating or preventing a GPCRX-associated disorder in humans. For example, the polypeptide and nucleic acid are useful for treating or preventing cardiomyopathy, atherosclerosis and disorders related to cell signal processing and metabolic pathway modulation. The antibody is useful for treating or preventing diabetes and disorders related to cell signal processing and metabolic pathway modulation (all claimed). The GPCRX polypeptide, nucleic acid and antibody are useful for the treatment or diagnosis of other GPCRX-associated disorders, e.g. bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease, multiple sclerosis, angina pectoris, myocardial infarction, ulcers, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders, the metabolic syndrome X, wasting disorders associated with chronic diseases, metabolic disorders, obesity, infectious disease, cancer-associated cachexia, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

ADMINISTRATION - The GPCRX nucleic acids, polypeptides and antibodies are administered parenterally, e.g. intravenous, intradermal, subcutaneous, oral, transdermal, transmucosal or rectal administration. No specific dosages are given in the specification.

EXAMPLE - The quantitative expression of various clones was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative polymerase chain reaction (PCR) (RTQ PCR; TAQMAN (RTM)). RTQ PCR was performed on a Perkin-Elmer Biosystems ABI PRISM (RTM) 7700

Sequence Detection System. Various collections of samples are assembled on the plates, and referred to as Panel 1 (containing cells and cell lines from normal and cancer sources), Panel 2 (containing samples derived from tissues, in particular from surgical samples, from normal and cancer sources), Panel 3 (containing samples derived from a wide variety of cancer sources), Panel 4 (containing cells and cell lines from normal cells and cells related to inflammatory conditions) and Panel CNSD.01 (containing samples from normal and diseased brains). First, the RNA samples were normalized to constitutively expressed genes such as beta--actin and GAPDH. RNA (approximately 50 ng total or approximately 1 ng polyA+) was converted to cDNA using the TAQMAN (RTM) Reverse Transcription Reagents Kit and random hexamers according to the manufacturer's protocol. Reactions were performed in 20 microlitres and incubated for 30 minutes at 48 degrees Centigrade. cDNA (5 microlitres) was then transferred to a separate plate for the TAQMAN (RTM) reaction using beta-actin and GAPDH TAQMAN (RTM) Assay Reagents and TAQMAN (RTM) universal PCR Master Mix according to the manufacturer's protocol. Reactions were performed in 25 microlitres using the following parameters: 2 minutes at 50 degrees Centigrade; **10 minutes** at 95 degrees Centigrade; 15 seconds at 95 degrees Centigrade/1 minute at 60 degrees Centigrade (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for beta-actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their beta-actin /GAPDH average CT values. Normalized RNA (5 microlitres) was converted to cDNA and analyzed via TAQMAN (RTM) using **One Step RT-PCR** Master Mix Reagents and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's Primer Express Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration 250 nM, primer melting temperature (Tm) range = 58-60 degrees Centigrade, primer optimal Tm = 59 degrees Centigrade, maximum primer difference = 2 degrees Centigrade, probe does not have 5' G, probe Tm must be 10 degrees Centigrade greater than primer Tm, amplicon size 75 base pairs (bp) to 100 bp. The probes and primers selected were synthesized by Synthegen. Probes were double purified by high pressure liquid chromatography (HPLC) to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM. Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate. PCR cocktails including two probes (a probe specific for the target clone and another gene-specific probe multiplexed with the target probe) were set up using 1 x TAQMAN (TM) PCR Master Mix for the PE Biosystems 7700, with 5 mM magnesium chloride, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold (TM), and 0.4 U/ml RNase inhibitor, and 0.25 U/ml reverse transcriptase. Reverse transcription was performed at 48 degrees Centigrade for 30 minutes followed by amplification/PCR cycles as follows: 95 degrees Centigrade for **10 minutes**, then 40 cycles of 95 degrees Centigrade for 15 seconds, 60 degrees Centigrade for 1 minute. The results from each panel could then be analyzed. (165 pages)

L8 ANSWER 3 OF 3 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2002-09257 BIOTECHDS
TITLE: Nucleic acids encoding secreted polypeptides, designated MOLX polypeptides, useful for treating a MOLX-associated disorder, e.g. cardiomyopathy, atherosclerosis, diabetes and metabolic disorders;
vector-mediated gene transfer, expression in host cell, agonist, antagonist and antibody for recombinant protein production, drug screening and gene therapy
AUTHOR: SPADERNA S K; TCHERNEV V; LIU X; SHENOY S; SPYTEK K; ZERHUSEN B; PATTURAJAN M; TAUPIER R J; RASTELLI L; GROSSE W M; SZEKERES E S; ALSOBROOK J; LEPLEY D M; SHEN L; BURGESS C E; SHIMKETS R A; PADIGARU M
PATENT ASSIGNEE: CURAGEN CORP
PATENT INFO: WO 2002006339 24 Jan 2002
APPLICATION INFO: WO 2000-US21249 3 Jul 2000
PRIORITY INFO: US 2001-279856 29 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-155038 [20]
AN 2002-09257 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Isolated nucleic acids encoding secreted polypeptides, designated MOLX polypeptides (i.e. a MOL polypeptide where X is an integer from 1 to 8), are new.

DETAILED DESCRIPTION - Isolated nucleic acids encoding secreted polypeptides, designated MOLX polypeptides (i.e. a MOL polypeptide where X is an integer from 1 to 8), are new. A nucleic acid (N1) encoding a MOLX polypeptide is selected from: (a) a nucleic acid encoding a mature form of a polypeptide selected from one of the 12 amino acid sequences (P1) defined in the specification; (b) a nucleic acid encoding a variant of a mature form of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the sequence of the mature form; (c) a nucleic acid encoding an amino acid sequence selected from P1; (d) a nucleic acid encoding a variant of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the amino acid sequence selected from P1; (e) a nucleic acid fragment encoding at least a portion of an amino acid sequence selected from P1, or a variant of the polypeptide, where one or more residues in the variant differs from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the amino acid sequence selected from P1; or (f) a nucleic acid comprising the complement of (a)-(e). INDEPENDENT CLAIMS are also included for the following: (1) an isolated polypeptide (P2) comprising an amino acid sequence selected from: (a) a mature form of a polypeptide selected from P1; (b) a variant of a mature form of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the sequence of the mature form; (c) an amino acid sequence selected from P1; (d) a variant of a sequence selected from P1, where one or more amino acid residues differ from the sequence of the mature form, provided that the variant differs in no more than 15% of the residues from the amino acid sequence selected from P1; (2) a vector comprising N1 operably linked to a promoter; (3) a cell comprising the vector of (2); (4) an antibody (Ab1) that immunospecifically binds to P2; (5) a method of determining the presence or amount of P2 in a sample, comprising contacting the sample with Ab1; and determining the presence or amount of Ab1 bound to the polypeptide, therefore determining the presence or amount of P2 in the sample; (6) a method (M1) of determining the presence or amount of N1 in a sample, comprising contacting the sample with a probe that binds to N1; and determining the presence or amount of the probe bound to the nucleic acid; (7) a method (M2) of

identifying an agent that binds to P2, comprising contacting the polypeptide with the agent and determining whether the agent binds to the polypeptide; (8) a method of identifying an agent that modulates the expression or activity of P2, comprising providing a cell expressing the polypeptide, contacting the cell with the agent, and determining whether the agent modulates expression or activity of the polypeptide, where an alteration in expression or activity of the polypeptide indicates that the agent modulates expression or activity of the polypeptide; (9) a method for modulating the activity of P2, comprising contacting a cell sample expressing P2 with a compound that binds to the polypeptide in an amount sufficient to modulate the activity if the polypeptide; (10) a method of treating or preventing a MOLX-associated disorder in a human, comprising administering P2, N1 or Ab1; (11) a kit comprising P2, N1 or Ab1; (12) a method for determining the presence of or predisposition to a disease, preferably cancer, associated with altered levels of P2 in a first mammalian subject; (13) a method for determining the presence of or predisposition to a disease, preferably cancer, associated with altered levels of N1 in a first mammalian subject; (14) a method of treating a pathological state in a mammal, comprising administering a polypeptide in an amount that is sufficient to alleviate the pathological state, where the polypeptide has a sequence at least 95% identical to a polypeptide comprising an amino acid sequence selected from P1, or its biologically active fragment; (15) a method of treating a pathological state in a mammal, comprising administering Ab1 in an amount that is sufficient to alleviate the pathological state; (16) a method for the screening of a candidate substance interacting with an olfactory receptor polypeptide selected from P1, or their fragments or variants; (17) a method for screening of ligand molecules interacting with an olfactory receptor polypeptide selected from P1; (18) a method for screening of ligand molecules interacting with an olfactory receptor polypeptide selected from P1.

WIDER DISCLOSURE - Also disclosed as new are: (1) antisense nucleic acid molecules hybridizable to or complementary to a sequence selected from one of the 12 nucleotide sequences defined in the specification; (2) fusion proteins comprising a MOLX polypeptide; and (3) a method for monitoring the effectiveness of treatment of a subject with an agent e.g. antagonist, agonist, polypeptide, etc.

BIOTECHNOLOGY - Preferred Polypeptide: P2, preferably, comprises a sequence of a naturally occurring allelic variant of a sequence selected from P1. The allelic variant comprises a sequence that is a translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from one of the 12 nucleotide sequences (N2) defined in the specification. The amino acid sequence of the variant comprises a conservative substitution. Preferred Nucleic Acid: N1 comprises a nucleotide sequence of a naturally occurring allelic variant. N1 encodes a polypeptide comprising an amino acid sequence of a naturally occurring variant. The nucleic acid differs by a single nucleotide from a sequence selected from N2. Preferably, N1 is selected from: (a) a nucleotide sequence selected from N2; (b) a nucleotide sequence differing by one or more nucleotides from a nucleotide sequence selected from N2, provided that no more than 20% of the nucleotides differ from the sequence; (c) a nucleic acid fragment of (a) or (b); (d) a nucleotide sequence comprising a coding sequence (C1) differing by one or more nucleotide sequences from a coding sequence (C2) encoding an amino acid sequence selected from P1, provided that no more than 20% of the nucleotides in C1 differ from the nucleotides in C2; (e) an isolated polynucleotide that is a complement of the nucleotide of (d); (f) a nucleic acid fragment of (d) or (e). N1 hybridizes under stringent conditions to a sequence selected from N2 or its complementary sequence. Preferred Antibody: The antibody is humanized or monoclonal. Preferred Method: In M1, the presence or amount of the nucleic acid is used as a marker for cell or tissue type. The cell or tissue type is cancerous. In M2, the agent is a cellular receptor or a downstream effector. The method

of (12) comprises: (a) measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and (b) comparing the amount of the polypeptide in the sample of step (a) to the amount of polypeptide present in a control sample from a second mammalian subject known not to have, or not to be predisposed to the disease, where an alteration in the expression level of the polypeptide in the first subject as compared to the control sample indicates the presence of or predisposition to the disease. The method of (13) comprises: (a) measuring the amount of the nucleic acid in a sample from the first mammalian subject; and (b) comparing the amount of the nucleic acid in the sample of step (a) to the amount of the nucleic acid present in a control sample from a second mammalian subject known not to have, or not to be predisposed to the disease, where an alteration in the expression level of the nucleic acid in the first subject as compared to the control sample indicates the presence of or predisposition to the disease. The method of (16) comprises: (a) providing a polypeptide selected from P1, or its peptide fragment or a variant; (b) obtaining a candidate substance; (c) bringing into contact the polypeptide with the candidate substance; and (d) detecting the complexes formed between the polypeptide and the candidate substance. The method of (17) comprises: (a) providing a recombinant eukaryotic host cell containing a nucleic acid encoding a polypeptide selected from P1; (b) preparing membrane extracts of the recombinant eukaryotic host cell; (c) bringing into contact the membrane extracts prepared at step (b) with a selected ligand molecule; and (d) detecting the production level of second messengers metabolites. The method of (18) comprises: (a) providing an adenovirus containing a nucleic acid encoding a polypeptide selected from P1; (b) infecting an olfactory epithelium with the adenovirus; (c) bringing into contact the olfactory epithelium (b) with a selected ligand molecule; and (d) detecting the increase of the response to the ligand molecule. Isolation: The MOL polynucleotides are isolated using standard genetic engineering techniques.

ACTIVITY - Dermatological; Antianemic; Hepatotropic; Antipsoriatic; Vulnerary; Neuroprotective; Osteopathic; Antiarthritic; Immunosuppressive; Cerebroprotective; Hemostatic; Vasotropic; Hypotensive; Antiinflammatory; Immunomodulator; Immunostimulant; Anti-HIV; Virucide; Antibacterial; Fungicide; Antirheumatic; Antiasthmatic; Cardiovascular-Gen.; Anticoagulant; Antiulcer; Ophthalmological; Nootropic; Antiparkinsonian; Anticonvulsant; Muscular-Gen.; Endocrine-Gen.; Aphrodisiac; Tranquilizer; Antidepressant; Analgesic. No biological data given.

MECHANISM OF ACTION - Gene therapy; MOLX antagonist; MOLX agonist. No biological data given.

USE - The MOLX polypeptide, nucleic acid and antibody are useful for treating or preventing a MOLX-associated disorder in humans. For example, the polypeptide and nucleic acid are useful for treating or preventing cardiomyopathy, atherosclerosis and disorders related to cell signal processing and metabolic pathway modulation. The antibody is useful for treating or preventing diabetes and disorders related to cell signal processing and metabolic pathway modulation (all claimed). The MOLX polypeptide, nucleic acid and antibody are useful for the treatment or diagnosis of other MOLX-associated disorders, e.g. chromosomal disorders, albinism, anemia, liver cirrhosis, psoriasis, scarring, neurodegeneration, osteoarthritis, organ rejection, cerebral thrombosis, ischemia, hypertension, systemic lupus erythematosus, immune diseases, immunodeficiency, HIV, viral, bacterial and fungal infections, hepatitis, rheumatoid arthritis, asthma, hematopoietic, thrombolytic, hemostatic diseases, thrombocytopenia, ulcers, ocular disease, Alzheimer's, Parkinson's and Huntington's diseases, muscular diseases, growth disorders, loss of libido, stress, depression, pain and epilepsy; for preventing chemotherapy side effects; and as a contraceptive.

ADMINISTRATION - The MOLX nucleic acids, polypeptides and antibodies are administered parenterally, e.g. intravenous, intradermal,

subcutaneous, oral, transdermal, transmucosal or rectal administration. No specific dosages are given in the specification.

EXAMPLE - The quantitative expression of various clones was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative polymerase chain reaction (PCR) (RTQ PCR; TAQMAN (RTM)). RTQ PCR was performed on a Perkin-Elmer Biosystems ABI PRISM 7700 Sequence Detection System. Various collections of samples are assembled on the plates, and referred to as Panel 1 (containing cells and cell lines from normal and cancer sources), Panel 2 (containing samples derived from tissues, in particular from surgical samples, from normal and cancer sources), Panel 3 (containing samples derived from a wide variety of cancer sources), Panel 4 (containing cells and cell lines from normal cells and cells related to inflammatory conditions) and Panel CNSD.01 (containing samples from normal and diseased brains).First, the RNA samples were normalized to constitutively expressed genes such as beta--actin and GAPDH. RNA (approximately 50 ng total or approximately 1 ng polyA+) was converted to cDNA using the TAQMAN (RTM) Reverse Transcription Reagents Kit and random hexamers according to the manufacturer's protocol. Reactions were performed in 20 microlitres and incubated for 30 minutes at 48 degrees Centigrade. cDNA (5 microlitres) was then transferred to a separate plate for the TAQMAN (RTM) reaction using beta-actin and GAPDH TAQMAN (RTM) Assay Reagents and TAQMAN (RTM) universal PCR Master Mix according to the manufacturer's protocol. Reactions were performed in 25 microlitres using the following parameters: 2 minutes at 50 degrees Centigrade; **10 minutes** at 95 degrees Centigrade; 15 seconds at 95 degrees Centigrade/1 minute at 60 degrees Centigrade (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for beta-actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their beta-actin /GAPDH average CT values. Normalized RNA (5 microlitres) was converted to cDNA and analyzed via TAQMAN (RTM) using **One Step RT-PCR** Master Mix Reagents and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's Primer Express Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration 250 nM, primer melting temperature (Tm) range = 58-60 degrees Centigrade, primer optimal Tm = 59 degrees Centigrade, maximum primer difference = 2 degrees Centigrade, probe does not have 5' G, probe Tm must be 10 degrees Centigrade greater than primer Tm, amplicon size 75 base pairs (bp) to 100 bp. The probes and primers selected were synthesized by Synthegen. Probes were double purified by high pressure liquid chromatography (HPLC) to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM. Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate. PCR cocktails including two probes (a probe specific for the target clone and another gene-specific probe multiplexed with the target probe) were set up using LX TaqMan (TM) PCR Master Mix for the PB Biosystems 7700, with 5 mM magnesium chloride, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold (TM), and 0.4 U/ml RNase inhibitor, and 0.25 U/ml reverse transcriptase. Reverse transcription was

performed at 48 degrees Centigrade for 30 minutes followed by amplification/PCR cycles as follows: 95 degrees Centigrade for **10 minutes**, then 40 cycles of 95 degrees Centigrade for 15 seconds, 60 degrees Centigrade for 1 minute. The results from each panel could then be analyzed.(223 pages)

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=> D HIS

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     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 10:32:18 ON 19 JUL 2005

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 10:32:54 ON 19 JUL 2005
L1       301500 S (RT-PCR OR REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION)
L2            1 S L1 AND (LESS (5A) 10 MINUTE###)
L3          153 S L1 AND 10 MINUT###
L4           13 S L3 AND (SINGLE TUBE OR TUBE OR VESSEL OR CARTRIDGE)
L5           13 DUP REM L4 (0 DUPLICATES REMOVED)
L6          478 S ONE-STEP RT-PCR
L7          197 DUP REM L6 (281 DUPLICATES REMOVED)
L8            3 S L7 AND (10 MINUTES)

=> TWO-stage RT-PCR
TWO-STAGE IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s TWO-stage RT-PCR
L9            0 TWO-STAGE RT-PCR

=> (single-step or two-stage or two-step) (5a) (RT-PCR or reverse transcriptase
polymerase chain reaction)
(SINGLE-STEP IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s (single-step or two-stage or two-step) (5a) (RT-PCR or reverse transcriptase
polymerase chain reaction)
L10         562 (SINGLE-STEP OR TWO-STAGE OR TWO-STEP) (5A) (RT-PCR OR REVERSE
                TRANSCRIPTASE POLYMERASE CHAIN REACTION)

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Connection closed by remote host
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